Effects of Crude Oils on Arctic Marine Invertebrates

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1. SUMMARY

Recent developments in the Canadian Arctic have made it increasingly clear that our present understanding of both short and long-term consequences of oil pollution upon Arctic marine ecosystems is far from adequate. It is generally conceded that recovery from a severe ecological disturbance would be a lengthy process in the Arctic. This study addresses itself primarily to the question of just how susceptible certain invertebrate components of the marine inshore ecosystem are to significant disruption by oil pollution.

Physical and biological variables that are likely to play an important role in modifying the ecological impact of a spill are discussed briefly. Marine organisms may encounter varying concentrations and forms of crude oil depending upon habitat and circumstance. Oil-animal interactions in three distinct habitats are considered, namely, the sub-ice, the neritic and the benthic habitats. Biological effects of three general types are examined; short term lethal effects, sublethal physiological effects and sublethal behavioral effects. Considerable variability in the responses of different species occurred in all three categories.

Lethal toxicity studies indicate a relatively high tolerance level for crude oil among most of the species examined. However, a closer examination of the more subtle sublethal effects suggests that the oil is not as benign as it first appears. Activity and metabolism may both be severely impaired by exposure to relatively low oil concentrations. Potential ecological consequences of such sublethal physiological effects are discussed.

Behavioral studies were carried out to investigate the responses of several species to the presence of crude oil masses, crude oil tainted food and crude oil contaminated sediment. None of the species examined were attracted by the oil. Some species such as *Onisimus* avoided oil masses, rejected oil-tainted food and preferentially selected uncontaminated over contaminated sediment. In contrast, other species such as *Mesidotea* were strictly neutral; there was no attraction to or repulsion from oil masses, tainted food was consumed almost as readily as untainted and the animals did not differentiate between contaminated and uncontaminated sediment. Possible long-term ecological consequences of these behavioral responses are discussed in detail.

2. INTRODUCTION

The potential for a major oil pollution incident in the arctic has increased dramatically over the past several years, leading Dunbar (1971) to suggest that oil pollution is now the most serious and immediate threat facing the arctic ecosystem. This is particularly true for the arctic marine ecosystem now that emphasis is rapidly shifting to offshore drilling in the coastal waters of the Beaufort Sea. The technological feasibility and potential hazards of such an undertaking are subjects of continuing debate and speculation. Particular concern has been expressed for climatological and ecological consequences of major submarine well blow-outs. The glaring inadequacy of our present understanding of both short and long-term effects of such accidents has been brought into sharp focus by this debate. Our ecological ignorance has made it difficult to realistically sustain or convincingly refute the many highly speculative predictions of widespread environmental disaster. As Dunbar (1971) so aptly puts it, "we have been caught in a state of scientific near nudity in the particular respect in which we now so urgently need protective covering; namely knowledge of what the proposed developments will do to the environment in precise terms, and knowledge of what should be done to conserve and protect."

This general uncertainty is unquestionably compounded by the fact that little reliable information is available about even basic ecology and physiology of the overwhelming majority of arctic marine species. This creates problems in the effective conduct and proper interpretation of experimental studies on interactions between crude oil and arctic organisms. Furthermore, it makes it especially difficult to assess the applicability, in the arctic context, of generalizations about oil-ecosystem interactions derived from numerous studies conducted in temperate and tropical marine areas.

At the outset it should be made clear that I do not propose to engage in detailed impact prediction. Even in temperate waters where intensive research has provided a solid information base, it is still impossible to predict, with any degree of confidence, the precise ecological effects of an anticipated oil spill. Even after a spill has occurred it has proven difficult to assess the overall environmental impact. Given the dearth of information about arctic marine ecosystems, any attempt at detailed impact prediction at present can only be considered an exercise in futility.

Ecological concern has thus far been centred upon those components of the ecosystem that are of direct present or potential economic importance to northern residents, particularly marine mammals and water fowl. Although one should not underestimate the importance of gaining

as complete an understanding as possible of direct effects of oil on these "high profile" animals, it would be particularly short-sighted and potentially rather dangerous, to confine attention to just these species. They do not and cannot, exist in a vacuum, but are part of a complex ecological web characterized by a multitude of vital interdependencies. Thus far little attention has been paid to those "low profile" organisms of little direct economic consequence that, nevertheless, form a critical part of the ecological web upon which higher forms are dependent. In an attempt to redress the balance somewhat, this study will focus primarily upon the effects of northern crude oils on arctic marine invertebrates.

It is generally conceded that recovery from a severe ecological disturbance, such as a major oil spill, would be a lengthy process in the Arctic. This is a consequence of the slow growth, extended life cycles and longer reproductive periodicity of Arctic animals (Chia, 1970) as well as of the slow dispersion and degradation of oil at low temperatures (Glaeser, 1971). However, it is still not clear just how susceptible various components of the Arctic marine ecosystem are to damage by oil pollution. It is to this primary question that the present study chiefly addresses itself. In view of the great complexity of potential interactions between oil and animal communities it would be manifestly impossible in a study of such limited duration to explore more than a few of the myriad aspects of the problem.

Unlike many other pollutants, crude oil is relatively insoluble in seawater. As a consequence, spilled oil does not spread uniformly through the environment but tends rather to concentrate in specific areas of the environment in a variety of forms. Marine organisms may thus encounter varying concentrations and forms of crude oil depending upon habitat and circumstance.

In this study I have attempted to examine some of the more important oil-animal interactions that are likely to occur in the coastal Beaufort Sea environment, namely, interaction with sub-ice oil lenses, with oil dispersed in particulate form in the water column and with oil trapped in bottom sediments.

Interaction of animals with oil in any of the above forms may result in three distinct levels of effect. Threshold concentrations for each of these levels of effect differ considerably. Generally one can distinguish:

(1) Short-term lethal effects: in which the animals succumb

rapidly. Usually oil concentrations in the high ppm or low ppt range are involved.

(2) Sublethal physiological effects: which do not result in rapid death of the organism but involve major modifications of a variety of physiological processes that may prove lethal in the long-term. Such effects generally become apparent at oil concentrations in the high ppb and low ppm range.

(3) Behavioral-integrative effects: which may not directly involve major physiological disruptions but are reflected in inappropriate behavioral responses to either biotic or abiotic features of the natural habitat, either as a direct effect in response to the presence of oil, or as an indirect effect resulting from the masking or mimicking of natural chemical cues by specific components of the oil. Concentrations in the low ppb range may be sufficient to evoke responses.

In this study I have considered aspects of all three levels of effects but have emphasized sublethal and behavioral effects in the belief that these are more likely to have greater long-term, and more widespread ecological significance.

3. THE NATURE OF THE THREAT OF OIL POLLUTION TO MARINE LIFE: A RESUME OF THE CURRENT STATE OF KNOWLEDGE

3.1 Problems in assessing oil impact in the Arctic

Oil pollution biology is a relatively new and active field as evidenced by the rapidly accumulating literature on the subject. Virtually all of the available information on the effects of crude oil on marine organisms and communities is derived from studies involving tropical and temperate ecosystems. The purpose of this review is not to provide an exhaustive survey of this growing literature, but rather to present a general conceptual framework upon which the meagre and fragmented information about oil effects in the arctic marine environment can be viewed to maximum advantage.

Few arctic marine oil spills have been studied in sufficient detail and for a long enough period to realistically assess the environmental impacts of such incidents. A complete absence of pre-spill baseline data has, in most instances, served to further compound the uncertainty. Impact prediction is rendered virtually impossible by the fact that little reliable information is available at present about even the most basic ecology and physiology of the great majority of arctic marine species.

Any discussion of oil impact in the marine arctic must necessarily, therefore, rely heavily upon information accumulated in the many excellent follow-up studies of oil spills in temperate seas, and in laboratory studies upon temperate species. However, considerable caution is required in using such information in an arctic context. Several features of the arctic marine ecosystem may have an important bearing upon the ecological consequences of an oil spill. That certain major crude oil spills in temperate waters (notably the Santa Barbara and Torrey Canyon incidents) were not as ecologically devastating as anticipated, should not make us overly complacent about the potential impact of comparable spills in the Arctic Ocean.

3.2 Factors influencing oil impact upon marine systems

3.2.1 The interacting variables

Studies on the impact of petroleum pollution upon marine ecosystems often yield conflicting or inconclusive results that make it very difficult to establish general principles. That this is so, is largely due to the very complex nature of the pollutant, both in its chemical and physical characteristics, and to the diverse physiological responses of different types of organisms to various components of the oil. Much of the difficulty in predicting oil spill effects is attributable to the fact that the ultimate fate of each spill is determined by several interacting variables each of which may play a

lesser or greater role in influencing the impact upon the ecosystem (Straughan, 1972). Several of these factors are common to arctic and temperate oil spills, others have important arctic aspects while some have features that are particularly unique in the arctic context. For convenience I have separated them into physical and biological categories.

A. Physical factors:

1. Type of oil
2. Dose of oil
3. Manner of introduction into the environment
4. Physiography and hydrography of spill area
5. Season of spill
6. Weather conditions
7. Method of treatment

B. Biological factors:

8. Season of spill
9. Adaptation effects
10. Synergistic effects
11. Nature of biota
12. Mode of interaction of oil with animals

It is interaction of all of these complex factors in the uniquely arctic marine environment that will determine the overall impact of any given oil spill. In some spills one or two of these factors may assume an overwhelming importance in determining the magnitude of the impact. I would like to briefly discuss these factors from the point of view of oil spills in arctic waters.

3.2.2 Type of oil

The type of oil spilled in a pollution incident clearly has a major bearing upon the magnitude of the resulting ecological impact. Petroleum pollutants generally fall into two very broad categories. Non-persistent pollutants include the lighter refined components such as gasoline, kerosene, diesel oil etc. These are volatile components that have generally been found to evaporate rather quickly following a spill. Persistent pollutants such as crude oil, bunker fuel etc. have a large bulk of essentially non volatile components that may remain in the environment for an indefinite period of time. In the absence of far northern refining facilities at present it is the latter type of pollutants that are the most serious threat

to arctic ecosystems. It is generally assumed that the toxicity of refined products is greater than that of crude oil, however, Butler and Berkes (1972) after an extensive review of the literature suggest that the available data does not warrant such a conclusion. Shelton (1971), however, maintains that apart from the light refined components, petroleum products are not highly toxic. This would also appear to be the general conclusion to be drawn from the extensive series of studies that followed upon the Torrey Canyon incident (Smith, 1970). However, toxic effects of crude oils have been widely reported (see review by Butler and Berkes, 1972), and Allen (1971) found that crude oils and heavier bunker oils have a significantly greater inhibiting effect upon cleavage of sea urchin eggs than do more highly refined petroleum products. Much of the confusion, no doubt, arises from the fact that different types of crude oil vary quite extensively in their toxic effects as Ottway (1971) has clearly shown for several species of intertidal organisms. Kuhnhold (1970) reports a similar phenomenon with regard to crude oil toxicity to herring eggs. Up to the present there has been no indication of the relative toxicity to arctic marine organisms of northern crude oils.

3.2.3 Dose of oil

That the quantity of oil spilled in a given incident markedly influences environmental impact is so obvious that little comment is required. It is anticipated that a blowout in the Beaufort Sea would release approximately 2500 barrels of oil a day into the environment with a gradual reduction to 1500 barrels a day. Particular concern has been expressed for a blowout occurring late in the drilling season, when rapidly forming ice cover would prevent drilling a relief well until the following ice-free season, some 10 months later (Pimlott, 1974). Simple arithmetic suggests that the total quantity of oil released during this period would be approximately equal to that spilled during the Torrey Canyon incident.

3.2.4 Manner of introduction of oil into the environment

The fact that the oil will be released into the water from the sea floor under very high pressure, rather than released relatively passively onto the sea surface, is likely to have important consequences in regard to the distribution of oil in various parts of the environment. A blowout scenario has been prepared (Beaufort Sea Technical Report #33) that provides a description of the likely behavior of the oil during,

and for some time after, release. The probable accumulation of oil in various parts of the environment and the consequent potential biological effects are discussed in section 6.

3.2.5 Physiography and hydrography of spill area

Coastal configuration, current flows, tidal conditions and physical characteristics of the water column will all play a major role in determining the magnitude of the area of the marine ecosystem affected by a major spill. The potential influences of these factors on an oil spill are considered in detail in Beaufort Sea Technical Reports #16, #17, #18, and #24.

3.2.6 Season of spill

That seasonal changes in certain physical parameters such as temperature or ice-cover may influence the impact of an oil spill is obvious. Not so obvious is the fact that the susceptibility of animals to certain pollutants may vary considerably with season as a consequence of changes in the physiological condition of the animal (Crapp, 1971). Nothing, however, is known about seasonal variations in the sensitivity of arctic animals to crude oil.

3.2.7 Weather conditions

Weather conditions at the time of a major spill, particularly such factors as wind speed and direction, will play an important role not only in determining the magnitude of the area affected but also, by influencing sea state, in determining the quantities of oil entrained in the water column. These aspects of the problem are considered in detail in Beaufort Sea Technical Reports #19 and #21.

3.2.8 Method of treatment

The manner in which spilled oil is treated during clean up attempts is an important feature in determining biological impact. This was one of the major lessons learned from the Torrey Canyon spill, where the use of dispersants appears to have caused more ecological damage than did the oil itself. Potential biological effects of the use of dispersents are discussed in section 6. Various clean up options available for use in the arctic are discussed in Beaufort Sea Technical Report #31.

3.2.9 Adaptation and synergistic effects

Adaptation and synergistic effects may subtly modify the impact of oil pollution on a given marine population. Studies in temperate regions suggest that some species adapt to continued exposure to low levels of petroleum and thus exhibit greater tolerance to more massive doses (Kanter et al., 1971). On the other hand, prior or simultaneous exposure of the animals to other types of pollutants may serve to increase the animals susceptibility to petroleum. In view of the relatively unpolluted state of the Arctic Ocean, neither pre-adaptation not synergistic effects are likely to play significant roles in modifying responses to oil spills.

3.2.10 Nature of the biota

Even a cursory examination of the considerable literature on the biological effects of oil pollution reveals a marked difference in the ability of different types of animals to tolerate the presence of oil in their environment. Even allowing for different types of oil and different preparative and experimental techniques one is, nevertheless, forced to the conclusion that certain species can thrive in incredibly high concentrations of oil while others species are killed or severely stressed by even a hint of petroleum in their environment. The precise reasons for this remarkable difference in tolerance are not at present known, largely due to the fact that we have no very clear idea of which components, and what mechanisms of toxicity, are responsible for irreversible damage to organisms exposed to a given crude oil. The following is a brief summary of a number of reports substantiating the above conclusion. It is by no means an exhaustive review of the subject, but provides some indication of the wide biological variability, a major factor in determining the overall impact of an oil spill upon a given ecosystem.

Data on sensitivities of various species to oil pollution have been derived from two different sources; field observations following accidental or controlled oil spills and laboratory toxicity studies conducted under standardized environmental conditions. The former are usually difficult to interpret because of the lack of controls and the potential involvement of a wide range of uncontrollable environmental variables that may modify the overall effect of the oil.

Studies of Mironov (1970) suggest that zooplankton may be particularly susceptible to crude oil. Although most species examined (mostly copepods) tolerated 1 ppm, almost all species experienced high mortality within 24 hours when exposed to 100 ppm oil. There is little firm field data

regarding zooplankton mortality in the vicinity of oil spills largely because of difficulties in assessing effects and a lack of adequate baseline information for the areas in question. According to Smith (1970) there appeared to be "little detected damage suffered by planktonic organisms in the western English Channel following the release of oil from the Torrey Canyon". However, larval stages of a wide range of marine invertebrates that at times constitute a substantial portion of the zooplankton community have in general been found to be very sensitive to petroleum (Wells, 1972; Chia, 1973; Mironov, 1969).

Considerably more information is available regarding the effect of oil on benthic invertebrates, although much of it appears contradictory. Intertidal organisms have received the most extensive studies largely because effects in the field are usually obvious and easy to assess and because such species inhabit areas that frequently bear the brunt of oil pollution incidents.

Sea anemones of the genus anthropleura are reported to be very resistant to oil pollution, even surviving heavy coating and smothering (Foster et al., 1971). In fact North et al. (1964) reports that not only did *Anthropleura xanthogrammia* suffer few ill effects from the Tampico Maru oil spill but that it occurs commonly in the effluent pools of a California refinery. However, other species of anemones appear to be very sensitive to oil (Manwell and Baker, 1967).

Molluscs too vary considerably in their response to oil. Thus successive immersion of oysters in oil caused little mortality, although sublethal deleterious effects were evident (Chipman and Galtsoff, 1949). Clams *Mercenaria mercenaria*, are reported to live in bays in Rhode Island the bottoms of which are literally "paved with oil" (Hawkes, 1961). The mussel *Mytilus galloprovincialis* is able to survive and function in high concentrations of oil although abnormal behavioral effects are evident at very high concentrations (Alyakrinskaya, 1966). In support of this Foster et al. (1971) reports that no significant mortality occurred among mussels, chitons and limpets following the Santa Barbara oil spill. However, not all bivalves are as tolerant of oil pollution as are these species. Large numbers of pismo clams and abalones were killed by the oil released in the Tampico Maru spill (North et al., 1964). A fuel oil spill on the U.S. west coast

resulted in the death of an estimated 300,000 razor clams in less than a week (Tegelberg, 1964). Gastropod molluscs are also susceptible to oil. The winkles *Littorina littorea* and *L. obtusata* experienced high mortalities when fresh crude oil was poured over them and then rinsed off (Crapp, 1969). Similar treatment resulted in high mortality in the limpet *Patella vulgata* (Nelson-Smith, 1968) and to the gastropods *Bittium reticulatum, Rissoa euxinica,* and *Gibbula divaricata* (Mironov, 1967).

Results for crustaceans are similarly variable, depending upon the oil type and nature of the exposure to the oil. Field experiments with barnacles suggest no obvious ill effects from exposure to crude oil (Crapp, 1969). However, the barnacle *Chthamalus fissus* suffered high mortality from smothering and heavily oiled gooseneck barnacles also died in the wake of the Santa Barbara oil spill. (Foster et al., 1971). That more than simple smothering may be involved is suggested by studies of Chipman and Galtsoff (1949) who found that some species of barnacles are killed by as little as 2% crude oil in less than three days. Lobsters and crabs suffered heavy mortality following the Tampico Maru spill (North et al., 1964). Large numbers of lobsters and other crustaceans were killed and washed up on the shore after a fuel spill in Buzzards Bay (Blumer et al., 1971). However, lobsters were not significantly affected by the Bunker C oil spilled from the Arrow in Nova Scotia (Scarratt, 1970). Large numbers of sand amphipods (Orchestoidae sp.) were oiled and killed in the Santa Barbara spill (Foster et al., 1970). Another sensitive amphipod rapidly succumbed in the presence of oil in the Buzzards Bay spill and could be used as an indicator of pollution. (Blumer et al., 1971). In marked contrast, the crab *Carcinus maenas,* along with the sea squirt *Ciona intestinalis* and the jellyfish *Aurelia aurita* are common in heavily oiled dock areas around Swansea (Naylor, 1965). Some coral species are sensitive to oil (Lewis, 1971) as are sea stars and sea urchins (Nelson-Smith, 1970). Marine worms, from the few studies that have been reported appear to be very tolerant of oil pollution (Foster et al., 1971; Blumer, et al., 1971).

On the basis of the above data it is difficult to draw hard and fast generalizations regarding the oil tolerance of various classes of invertebrates. Difficulties in making comparisons arise from the fact that differences in the tolerance of species are obscured by differences in the oils involved, differences in method of application of the oils, differences in the degree of weathering of the oil, in addition to differences

in a variety of other environmental parameters.

3.2.11 Mode of interaction of oil with marine organisms

The manner in which spilled oil interacts with organisms is an important factor in the overall impact of an oil spill. Crude oil, unlike many other pollutants, by virtue of its relative insolubility in seawater, does not spread uniformly through the environment from its point of release. The dose of oil to which a given population of animals will be exposed varies considerably, and often unpredictably, in different parts of the marine ecosystem. This aspect of the problem will be explored more fully in section 6 where detailed consideration will be given to the potential biological effects of oil dispersed in the water column, trapped in bottom sediments and accumulated at the ice-water interface.

3.3 Biological effects of crude oil

3.3.1 General biological and ecological effects

Blumer (1970) in reviewing some of the potential biological effects of oil pollution in marine systems suggested that adverse effects upon organisms can be conveniently grouped into the following general categories:

1. Direct kill of organisms by physical effects such as smothering.
2. Direct kill of organisms by contact toxicity, i.e. by components that have low solubility in seawater.
3. Direct kill of organisms by seawater soluble compounds.
4. Destruction of sensitive larvae and eggs.
5. Destruction of food sources.
6. Sublethal effects resulting in direct long-term mortality or in reduced tolerance to normal stresses.
7. Interference with subtle behavioral and integrative mechanisms of individuals, populations and communities.
8. Incorporation and possible concentration of carcinogens and other potentially toxic compounds in the food chain.

3.3.2 Short-term lethal effects

The greatest emphasis thus far has been placed upon short-term lethal effects of the types outlined in categories 1-4 above. Toxicity tests have usually involved either immersing the animals in whole oil for specific periods and then returning them to clean seawater for observations, or more realistically, exposing groups of animals to seawater dispersions or water soluble extracts of crude oil for specific time periods (usually 96 hours).

As pointed out, adverse effects of contact with crude oil

masses may arise from two distinct causes; a purely physical impairment of function as a consequence of the high viscosity of the oil and the chemical disruption of physiological functions resulting from the presence of low solubility toxic components in the oil. The purely physical effect may involve interference with respiratory exchange by clogging gill chambers, forming a film over gill surfaces or immobilizing appendages essential for ventilation. Simple immobilization of the animal in the oil mass may eventually cause death. Such physical smothering effects are a particular hazard to intertidal communities, where they have been studied intensively. Similar adverse effects might be expected in sub-ice communities.

Little is known about the specific nature of contact toxicity. Many studies have shown high mortalities of various species immersed in crude oil for short periods and then washed and transferred to clean seawater (Crapp, 1969; Nelson-Smith, 1968; Mironov, 1967). Many of the species that showed considerable susceptibility to this treatment were molluscs, supposedly equipped with a protective shell. In most such studies it is impossible to determine whether the animals are succumbing from physical or chemical causes. Crapp (1971), however, found that immersion of several species of molluscs in fresh crude oil led to high mortality, while similar exposure to weathered crude oil residue did not lead to significant mortality. This appears to rule out a physical cause, and indicates that a toxic component is responsible for the mortality and that it is a light volatile component that is lost on weathering.

Mortality following exposure to seawater dispersions of oil may similarly be attributable to physical or chemical factors, while in the case of exposure to water soluble extracts of the oil mortality is clearly attributable to chemical toxicity. In practice it is generally difficult to distinguish chemical from physical effects and soluble component toxicity from whole oil contact toxicity.

The different components of crude oil differ markedly in their degree of toxicity. Much of the toxicity appears to be associated with certain of the aromatic fractions boiling below 149°C (Ottway, 1971). The numerous studies conducted on the toxicity of various isolated components of crude oil have been reviewed by Butler and Berkes (1972). A number of generalizations may be drawn from these studies. Toxicity generally increases along the series paraffins, naphthenes, olefins and aromatics. Within a given series smaller molecules tend to be more toxic than larger, thus

octane and decane are very toxic, while dodecane and higher paraffins are virtually non-toxic (Van Overbeek and Blondeau, 1954). Unsaturated hydrocarbons naphthenic acids and compounds containing aromatic groups contribute to the total toxicity. Russian work, quoted by Galtsoff (1936) indicates that hexhydrobenzoic acid is one of the highly toxic components of Baku crude. Toxicity studies on various other refined constituents of crude oil have been reviewed by Nelson- Smith (1971). Considerably more work is required on the lethal and sublethal effects of other components of crude oils, particularly those components that are of a persistent nature.

The very complex and highly variable composition of crude oils accounts for the considerable differences in toxicity observed for various crude oils. The complexity of composition and wide variability in the solubilities of the different components also accounts for the difficulty encountered in conducting meaningful toxicity tests; one is never very sure of just how much of which types of toxic compounds are actually getting to the organism. The results of certain short-term lethal effect studies have already been reviewed in section 3.2.7

3.3.3 Sublethal physiological effects

Recently there has been a growing awareness that short-term lethality is an overly gross criterion for assessing ecological effects of pollutants. Useful as 96-hour bioassays have been in investigating certain aspects of pollution impact, they can never be considered as more than crude first approximations. Increasingly, emphasis is shifting to a variety of subtle sublethal effects that impair the organisms ability to function effectively. Prolongation of such impairment may in time lead to the reduction or elimination of populations in the affected area. The physiological effects are often difficult to detect and only recently has there been a general appreciation of the many insidious forms they may assume. Warner (1965) and others have suggested that pollution biologists should be more concerned with a "toxic response syndrome", a complex of diverse behavioral, physiological and biochemical parameters.

Metabolic rate has been widely employed as a measure of general physiological well-being and it can serve as a sensitive monitor of subtle changes in physiological state during exposure to environmental stresses. A glance through the admittedly scanty literature suggests that with regards to

the effects of oil on metabolism we are dealing with a rather complex situation. Oil has been shown to both enhance (Gilfillan, 1972; Hargrave and Newcombe, 1973) and depress (Avolizi and Nuwayhid, 1974; Dunning and Major, 1974) metabolic activity. As will be discussed in section 6, the apparent diversity in response may be more a reflection of a lack of appreciation of a variety of modifying factors, both physical and biological, rather than of an inherent species variability in response.

Activity (both locomotory and non-locomotory) is another useful measure of an animal's physiological well-being. Although it has been extensively used to monitor effects of a wide range of pollutants it has been virtually ignored in studies on oil pollution effects. That exposure to oil can modify activity has been demonstrated by Dunning and Major, 1974 (inhibition of ciliary activity in bivalves) and by Hargrave and Newcombe, 1973) (enhancement of crawling rate in gastropods) but at present the data is too scanty to permit meaningful generalizations.

3.3.4 Sublethal behavioral effects

There is limited but convincing evidence that crude oil released into the environment not only disrupts normal physiological functions of individuals but may also interfere with complex behavioral patterns necessary for effective integration of the various components of an ecosystem. This may be a consequence of a direct response to the presence of oil or an indirect effect resulting from the masking or mimicking of natural chemical cues by certain soluble components of the oil. Kittredge (1973) has pointed out that effects of the latter type could impair the organisms ability to locate food, a suitable niche or sexual partners. Before further advances can be made in this area by pollution biologists it will be necessary to have much more comprehensive information on the utilization of natural chemical cues by marine organisms.

The present study is restricted primarily to a consideration of direct attraction-repulsion responses to crude oil in a variety of forms in which it may occur in the environment. This is an important consideration in evaluating oil impact because as pointed out earlier spilled oil is not distributed uniformly through the marine environment. The behavioral response may play a significant role in determining the concentrations of oil to which the animals are exposed. Little reliable information is available at present regarding

this particular facet of the problem. A number of field observations are suggestive but of uncertain significance. Reports quoted by Nelson-Smith (1971) indicate that some fish species tend to avoid spilled oil floating on the sea surface. Blumer (1970) notes that certain purified hydrocarbons derived from kerosene attract lobsters, and he suggests that the massive mortality experienced by this species following the Buzzards Bay oil spill may in part be attributable to the fact that animals were attracted away from their normal food in the direction of the spill. Wilder (1970) observed that lobsters readily consume fish heavily contaminated with Bunker C oil. Unfortunately no observations were made to determine if the contaminated food was eaten more readily than clean food. In connection with the Arrow spill of Bunker C, Thomas (1970) reported that periwinkles appeared to migrate from heavily oiled areas to adjacent clean areas. Furthermore, he noted that clams, *Mya arenaria* generally moved out of oil polluted burrows. It is not clear whether this represented an aversion to the oil or a simple attempt to escape suffocation in the burrows. Definitive laboratory studies on the behavioral responses of marine organisms to petroleum and petroleum products are clearly lacking.

4. MATERIALS AND METHODS

4.1 Study areas and animal collections

Organisms employed in this study were collected in several general areas. The amphipods *Onisimus affinis*, *Corophium clarencense*, and *Atylus carinatus*, the cumacean *Brachydiastylis resima*, the barnacle *Balanus crenatus*, the sculpin *Myoxocephalus quadricornis* and the jellyfish *Halitholus cirratus* were collected in the vicinity of the Arctic Biological Station's field laboratory near the entrance to the Eskimo Lakes (approximately 69°25'N, 131°16'W). These so called lakes are in actual fact an interconnected complex embayment joined by a narrow channel to the base of Liverpool Bay. The lakes are brackish, with a salinity of about 15-17‰ in the vicinity of the collecting site. The water has an annual temperature range from about -1°C to +9°C.

Onisimus was collected in modified minnow traps baited with fish. *Corophium*, *Atylus*, *Brachydiastylis* and *Balanus* were carefully screened from mud samples obtained with a Peterson grab or a lightweight dredge. The medusa *Halitholus* is rather delicate, and specimens collected with a plankton net were invariably in poor condition. All medusa used in this study were collected in shallow water by scooping them up individually in beakers. Young sculpins (approx. 3 cm long) were collected in a similar manner. During 1974 the isopod *Mesidotea entomon* was collected in about 10 meters of water in Kugmallit Bay in the vicinity of Tuktoyaktuk by trawling from the M.V. Salvelinus. During the summer of 1975 collections of *Mesidotea entomon*, *M. sibirica* and *M. sabini* were obtained by trawling from M.V. Salvelinus in Thetis Bay to the east of Herschel Island. The copepod *Calanus hyperboreas* was collected in limited quantities by means of vertical plankton tows through leads in the Beaufort Sea pack ice (Station 13 - Grainger, 1975).

All animals were held in a recirculating seawater system at the field laboratory in a temperature and salinity regime approximating that in the natural habitat. For some of the winter studies collections of *Onisimus* were transported to Ste. Anne de Bellevue where they were maintained in a high capacity, refrigerated recirculating, seawater system until use (temperature 1°C, salinity 16‰).

4.2 Crude oils employed in Study

Three northern crude oils were employed in various phases of this study. The oils and values for certain physical parameters

(Keevil and Ramseier, 1975) are as follows:

Oil	Pour Point °C	Specific gravity	Viscosity centipoise
Pembina	-10	0.857	13
Atkinson Point	-45	0.919	61
Norman Wells	-50	0.833	8.5

A southern oil, Venezuela crude (Tijuana light) that has been extensively employed in biological studies in temperate areas was used for comparative purposes in certain of the studies.

To minimize loss of volatile components from the original sealed stock container as a result of frequent sample removal, aliquots of the oil were transferred to a large number of 2 ml screw cap vials. The tightly sealed vials were stored at 0° to 5°C, and a fresh vial was used for the preparation of each dispersion.

4.3 Fluorescence spectroscopy assay for crude oil

The quantity of crude oil present in dispersed form in seawater was determined spectro fluorometrically using the method of Keizer and Gordon (1973). A 100 ml subsample of the seawater was extracted twice with 10 ml aliquots of methylene chloride. The extracts were combined and the methylene chloride removed under vacuum at 31°C in a rotary evaporator. The residue was dissolved in 20 ml of spectrophotometric grade hexane. Fluorescence of the sample was measured in a Turner model 340 spectro fluorometer using a slit width of 15 nm, an excitation wave length of 325 nm and an emission wave length of 425 nm. Oil concentrations were read directly from calibration curves that were prepared at regular intervals.

4.4 Preparation of standard oil in seawater dispersions

A series of preliminary studies were conducted on several parameters associated with the experimental formation of oil in seawater dispersions. The relationship between the original concentration of oil added to the seawater and the concentration that remains in the form of a semi-stable dispersion was examined. A study was also made to determine the rates at which the various oils separate from the seawater to form surface slicks. The effect of temperature on the quantity of oil entrained in a seawater dispersion was also examined.

On the basis of the results of the preliminary dispersion studies the following standardized procedure for preparation of oil in seawater dispersions was chosen.

Four fixed levels of crude oil were selected for the studies. These were designated:

1. Control: seawater only, no oil.
2. Light: addition of 25 µl of crude oil per 500 ml of seawater.
3. Medium: addition of 250 µl of crude oil per 500 ml of seawater.
4. Heavy: addition of 1000 µl of crude oil per 500 ml seawater.

In all cases, the seawater was filtered through a 1µ or finer filter prior to adding the oil. The initial preparative steps were carried out at 20°-22°C. For the light and medium concentrations the required quantity of fresh crude oil was added to 500 ml of filtered seawater in a 32 oz. glass jar. The jars were tightly capped, placed on a reciprocating shaker and agitated at high speed (280 excursions per minute) for one hour. For the heavy oil concentrations the oil was added to 500 ml of filtered seawater and dispersed in a Waring blender at high speed (10,000 rpm) for 5 minutes. The oil-seawater dispersions were then transferred immediately to 500 ml separatory funnels and allowed to stand undisturbed for varying periods depending upon the particular oil type (Atkinson Point crude: 210 minutes; Venezuela and Pembina crude: 120 minutes; Norman Wells crude: 90 minutes). After standing, the lower 450 ml of seawater containing oil in a semi-stable dispersion was drawn off into 24 oz. glass jars. The final 50 ml containing coalesced oil was discarded.

In those studies in which the dispersant corexit 8660 was used the dispersant was added to the seawater at the same time as the oil in a 1:1 ratio; all other steps of the procedure were identical to those described above.

4.5 Exposure of animals to crude oil/seawater dispersions

The jars containing the oil in seawater dispersions, prepared as outlined above, were placed in an 8°C water bath and temperature-equilibrated prior to addition of the experimental animals. Pasteur pipettes attached to an air source were placed in each jar and provided a uniform, continuous stream of small bubbles for aerating the water. Animals were not fed during oil exposure periods which varied in duration, depending upon the particular experimental protocol.

4.6 Short-term toxicity tests

4.6.1 Exposure to oil in seawater dispersion

Groups of animals, ranging in size from 5 to 20 individuals

depending upon the species, were placed in the above described exposure jars. These animals had first been acclimatized at 8°C for several days to avoid stress. Usually all runs were conducted in duplicate. Animals were transferred to fresh temperature-equilibrated oil-seawater dispersions every 24 hours. At the time of transfer dead animals were counted and removed. Crustaceans were considered dead when they failed to exhibit readily detectable limb movement, while for *Halitholus* the criterion of death was failure to contract either spontaneously or when lightly stimulated. Each exposure test was continued for 96 hours. Results are expressed as percentage mortality at 24, 48, 72 and 96 hours using the combined data for each duplicate group. Routinely oil-toxicity tests were conducted at 8°C in seawater of 17‰ salinity, except for those with the planktonic copepod *Calanus* which were conducted at 5°C in seawater of 34‰ salinity.

An additional series of toxicity tests was conducted on *Onisimus* to examine the effect of temperature on dispersed oil toxicity. In these tests oil dispersions were prepared at either 0°C or at room temperature (22°C) and animals were exposed to the dispersions at 0°C or 8°C.

4.6.2 Exposure to oil contaminated sediments

Superficial sediments collected with a grab in the Eskimo Lakes were experimentally contaminated with varying quantities of Norman Wells, Pembina and Atkinson Point crude oils. Filtered seawater (500 ml) together with 10 gm of dried finely ground sediment were placed in each 32 oz screw cap jar. Oil was added to the jars in the following quantities: 0 ml (control), 0.25 ml (moderate) and 1.0 ml (heavy). The jars were shaken on a reciprocating shaker at a rate of 280 excursions per minute for 1 hour. The sediment was permitted to settle in the jar for 2 hours. The sediment along with approximately 250 ml of the supernatant was siphoned from beneath the surface oil slick into a flask and settled for an additional 2 hours. The supernatant was carefully decanted off and the damp sediment divided equally between 2-500 ml glass storage dishes. Seawater (250 ml) was added to each dish, using a watch glass to minimize disturbance of the sediment. The storage dishes were placed in constant temperature rooms at either 0°C or 10°C and allowed to temperature equilibrate for 1 hour. Adult *Onisimus affinis*, acclimated to the experimental temperature for several hours were then added in groups of 20 to each storage dish. Duplicate dishes were run for each oil level at each temperature. The storage dishes were fitted with loose glass covers. The dishes were checked daily and dead animals recorded and removed. Animals were not fed (other than the sediment) during the test period. After 10 days remaining animals were removed and the dishes and contained sediment were

left undisturbed at the same temperatures for approximately 7 days. Then, the supernatant seawater was carefully siphoned off and fresh seawater added. Groups of 20 fresh animals were then added to each of the dishes and mortality was monitored for a further 10 days as before. This cycle was repeated until a total of four groups of animals had been exposed to each sediment sample. The total mortality in each dish over each 10 day exposure was expressed as a percentage. In graphing the results each 10 day cumulative percent mortality was plotted at the mid-point of the respective exposure period.

4.7 Metabolic rate determinations

4.7.1 Intact animal metabolism

Metabolic rate studies were conducted on animals exposed for various periods to standardized oil dispersions as described above. Respiration rates were measured with a Gilson submarine respirometer using standard 15 ml Warburg flasks. One to 5 animals, depending upon the species, were placed in each flask along with 5 ml of filtered (1μ) seawater at a salinity similar to that at which the animals were collected. The centre well in each flask was charged with 0.2 ml of 20% KOH and a filter paper wick to absorb CO_2. Air was used as a gas phase throughout. The shaking rate was 72 oscillations per minute. Flasks were equilibrated for 45-60 minutes and readings were taken at 1 hour intervals over a period of 5-6 hours. Upon completion of the run animals were rinsed in distilled water, dried at 70°C for 24 hours and weighed. Respiration rates were calculated from the slope of the line obtained by plotting cumulative oxygen uptake against time. Metabolic rates are expressed throughout as μl O_2/mg dry weight/hr. Means and standard errors of the means were calculated for each group.

4.7.2 Cell free homogenate metabolism

Metabolic rates were determined for cell free homogenates prepared from adult *Onisimus* that had been exposed to standard dispersions of Norman Wells crude for 24 hours at 5°C. For each sample 12 animals were homogenized for 2 minutes in a Potter-Elvejem tissue homogenizer held in an ice bath with a motor driven pestle. The homogenization medium consisted of 3 ml of the sucrose base medium employed by Peterson and Anderson (1969) supplemented with yeast extract (10 gm/liter). The chilled homogenate was transferred to the Warburg flask and the homogenizer was rinsed with a further 2 ml of medium. The metabolic rate was determined at 14°C. All other experimental conditions

were as outlined above for intact animal metabolism.

4.8 Activity measurements

Onisimus affinis and *Halitholus cirratus* were used in a study of the effects of pre-exposure to crude oil emulsions on activity. It was necessary to define a readily quantifiable activity function for each species. Locomotory activity of *Onisimus* was measured by placing animals individually in a simple test chamber. This consisted of a shallow, circular pan (20 cm diam; 4 cm deep) in the centre of which was placed a second smaller pan (10 cm diam.) to form a peripheral annular "moat". This "moat" was subdivided into 16 equal parts by a series of lines radiating from the centre of the larger pan and cutting across the "moat" at right angles. This device is similar to that employed for amphipod activity studies by Mackintosh (1973). The chamber was held in a water bath at 8°C and the water in the moat was changed frequently. Animals were placed in the "moat" and left undisturbed for three minutes before monitoring of activity started. Activity was measured by counting the number of line crossings by the animals during two consecutive one minute periods and taking the mean. It is possible to obtain a rough estimate of average rate of locomotion in cm/min by multiplying the mean line crossings per minute (LPM) by 2.5 cm, the distance between the lines. However, for the purpose of the present study it was sufficient to express results as lines per minute. Activity of the various test groups (10 animals per group) was measured after exposing the animals to the standard oil dispersions at 8°C for 24 hours.

Rhythmic swimming pulsations of medusae have been extensively used as a measure of locomotory activity (Gatz et al., 1973). However, although rate of bell pulsations of *Halitholus cirratus* is extremely regular and a sensitive monitor of activity under normal conditions (see Appendix Table 26) it was found to be of limited use in the present study. Following the standard 24 hour period in control exposure jars without food the majority of the animals exhibited a characteristic feeding response. This involved several regular pulsations to bring the animal to the surface followed by a slow passive descent through the water column with tentacles spread horizontally. Under these circumstances it was difficult to obtain consistent pulsation rates. In cases where regular pulsation rates were measurable they were recorded. Routinely, however, activity was defined in terms of an activity score. Experimental groups were observed for 10 minutes and activity scores assigned to each individual as follows:

2 - animals pulsing regularly and exhibit swimming activity i.e. lift off the bottom of the test tank.

1 - animals pulsate with varying degrees of regularity but exhibit no swimming activity.

0 - animals remain quiescent on the bottom of the tank, except for slight irregular, uncoordinated twitches of the vellum.

For the 24 hour oil exposure tests the animals were held under conditions identical to those outlined in section 4.5. Activity was monitored before exposure, after 24 hour exposure and again after a 24 hour recovery period in clean seawater.

An additional series of experiments were conducted with *Halitholus* to determine the rate of onset of activity disruption. For each run five standard medium oil dispersions in 32 oz jars and five control jars containing clean seawater were held in a water bath at 8°C. Two animals were placed in each jar and the activity score determined (as outlined above) at hourly intervals for 6 hours. The oxygen concentrations in the exposure jars did not decline significantly during the 6 hour test period.

4.9 Behavioral responses to the presence of crude oil

4.9.1 Responses to oil masses

The procedure for measuring the attraction or repulsion of animals for oil masses was essentially similar to that employed by Percy (1974). Test chambers consisted of shallow pans subdivided into four equal zones (designated A, B, C and D) by lines inscribed on the bottom. These were immersed in an ice bath to maintain the seawater between 2° and 4°C. In order to record the distribution of the animals in the various zones at intervals, a camera equipped with a flash attachment was positioned so that the entire interior of the chamber was included in the field of view. For each run one zone was designated as an oiled zone and the remaining three as unoiled, or control zones. To hold the oil, small squares of sponge measuring approximately 2 cm x 2 cm x 1 cm were affixed to glass microscope slides with non-toxic silicone cement. Immediately prior to the run 20 animals were placed in the chamber and distributed approximately equally among the four zones. Then 1 ml of fresh crude oil of the desired

type was placed on a sponge square. As soon as most of the oil was absorbed the sponge was rinsed rapidly in seawater to remove excess oil. The oiled sponge was then placed in the centre of the pre-selected oiled zone. Similar sponge squares that had not been treated with oil were placed in the centre of each of the control zones.

To eliminate outside disturbance and to prevent the animals from visually distinguishing between the dark-coloured oiled sponges and the paler control ones, the entire apparatus was enclosed in a light-proof hood. Each run lasted for 30 minutes, with the chamber being photographed at two minute intervals. Four runs with a single species and a single oil type constituted a series, with each of the four zones being successively designated as the oiled zone. At the end of each run the chamber was thoroughly cleaned and refilled with fresh seawater. A freshly oiled sponge square was used for each run. The numbers of animals within each zone at each observation period were counted from the film negative and tabulated.

For each run the numbers of animals in the oiled zone at each observation were added to give an observed frequency for the oiled zone designated O'_z where z represents the particular oiled zone for a given run. The numbers of animals in each of the three unoiled zones were similarly added and the totals for the three zones combined to yield an observed frequency for the unoiled control zones designated O_u. For each run O'_z and O_u were combined to yield the total number of counts for that run. Statistical probability dictates that in the absence of an attraction or repulsion response, for each run, one quarter of the counts should occur in the oiled zone and three quarters in the unoiled zones. The total counts for a given run were divided accordingly to yield an expected frequency for the oiled zone, designated e'_z. The X^2 value was calculated from the observed and expected frequencies for each run. The X^2 values for each of the four runs in a given series were added to yield a series X^2 value (Spiegel, 1961) and the probability of the observed distribution being significantly different from a random distribution was determined.

In order to express the attraction or repulsion response in a clear and concise manner an affinity coefficient was defined as follows:

$$A.C. = \frac{\Sigma O'_z - \Sigma e'_z}{\Sigma e'_z} \times 100$$

where O'_z is the sum of the observed counts in the oiled zone for each of the four runs in a series ($\Sigma O'_z = O'_a = O'_b = O'_c + O'_d$) and $\Sigma e'_z$ is the sum of the expected counts in the oiled zone for each of the four runs in a given series ($\Sigma e'_z = e'_a + e'_b + e'_c + e'_d$)

An A.C. of zero indicates a neutral response and the animal is neither attracted or repelled by the oil. An increasingly positive value indicates an increasing degree of attraction, to a maximum of 100 which indicates a total attraction to the oiled zone. Similarly, an increasingly negative value indicates an increasing degree of repulsion, to a minimum of -100 which indicates a total repulsion from the oiled zone. The significance of the A.C. for each series was determined from the X^2 values calculated as indicated above.

4.9.2 Responses to oil-tainted food

The response of animals to oil-tainted food was examined in two ways. In the first, the animals were given a choice between oil-tainted and untainted food, while in the second case only tainted food was available to them. The food consisted of 1 cm blocks of fish which were tainted by immersing them in fresh oil at room temperature for one hour then rinsing them in seawater to remove excess oil. The same chamber as that used in the affinity study was filled with seawater. A block of tainted fish was placed in one of the zones and a similar block of untainted fish placed in the diagonally opposite zone. Ten to 20 animals (depending on the species) were introduced into the chamber mid way between the two food squares. The chamber was observed at two minute intervals and the number of animals in contact with each of the fish squares was noted. The observations continued for 20 - 30 minutes. The counts for all of the observations were summed and the results expressed as percentages of the counts for contact with the tainted or untainted food relative to the total counts. For each oil and each species the tests were repeated three times with fresh groups of animals. A similar procedure was adopted for the second part of the study except that the animals were presented only with tainted food or only with untainted food (control). Again, each run was repeated three times with fresh animals, using a new control group for each oil. Results are expressed as a percentage of the maximum possible score (i.e. all animals in contact with the food at every observation period is scored as 100%).

4.9.3 Response to oil tainted sediments

Oiled sediments were prepared as outlined in section 4.6.2, except in this instance 15 gm of ground dried sediment was added to each jar along with the following quantities of crude oils: Control, 0 ml; light, 0.05 ml; moderate 0.5 ml; heavy, 1.0 ml; extra heavy 2.0 ml. Following the final decanting, the sediment in each jar was divided equally among 3 disposable 100 mm petri plates which were then filled completely with clean seawater and covered. The prepared plates were placed around the periphery of the bottom of a circular 30 cm diameter polyethylene chamber filled to a depth of 10 cm with seawater. Six plates of sediment were placed in each of four such chambers; three of the plates contained uncontaminated sediment and three oil-tainted sediment each of the same oil concentration. Clean and contaminated sediment plates were placed alternately around the periphery. Four chambers were run simultaneously each containing one of the four levels of oil tested. The chambers were covered and held at a temperature of 5°- 8°C during the test. After the chambers had stood undisturbed for 1 hour a covered petri plate containing a group of the test organisms was placed in the centre of the circle of sediment containing plates. Groups of 100 *Onisimus*, 20 *Mesidotea* or 60 *Corophium* were placed in each test chamber. The covers of the sediment plates were carefully removed. Then the covers of the animal-containing plates were also removed, releasing the animals into the chamber. The chambers were covered and left undisturbed for 1 or 2 hours. At the end of this time the covers were carefully replaced on each of the sediment petri plates thus trapping animals burrowed in or crawling upon the sediment. Animals in each plate were screened from the sediment and counted. Animals remaining free in the chamber were also counted. The petri plates were discarded after each test and the chambers thoroughly cleaned. The numbers of animals in the three control plates and three tainted sediment plates in each chamber were each summed and the results expressed as a percentage of the total number of animals in all six plates. The X^2 test was used to determine the significance of the animal distribution in each chamber.

A further test was run with *Onisimus* using aged contaminated sediment. The oiled sediment was prepared as above and transferred to petri plates. The petri plates

without covers were placed in a circulating seawater tank and left undisturbed for 1 week. The control and tainted sediment plates were then transferred to the test chambers and treated exactly as outlined above.

5. RESULTS

5.1 Preliminary fluorescence studies

5.1.1 Fluorescence spectra of selected crude oils

Uncorrected fluorescence excitation and emission spectra for the four oils used in the study are presented in Figures 1 and 2, respectively. Spectral profiles for all of the oils are essentially similar with only minor differences in amplitude, form and wavelength of emission and excitation maxima. The excitation spectra all exhibit slight shoulders in the vicinity of 310 nm, although these tend to be more pronounced in scans of Pembina and Atkinson Point crudes. Excitation and emission wavelengths of 325 nm and 425 nm, respectively, were selected for routine oil assays.

Calibration curves for all four oils were essentially similar in form; a typical curve is shown in Figure 3. Most of the oil concentrations used in this study fell within the linear portion of the curve. At concentrations in excess of 0.5µl oil/ml of hexane severe quenching of fluorescence occurred. Routinely, samples were diluted several percent with hexane after the initial reading and then re-read to establish unequivocally that the correct side of the peak was being read. Calibration curves were prepared at frequent intervals, particularly after any adjustments in instrument setting.

5.1.2 Formation of oil in seawater dispersions

In order to establish a series of standard semi-stable oil dispersions with a range of crude oil content it was first necessary to examine the influence of several parameters on the formation of oil in seawater dispersions. These included the following:

5.1.2.1 The relationship between the quantity of oil added and the final concentration in a dispersed form

Clearly, the quantity of oil that remains entrained in a semi-stable dispersion does not increase indefinitely as the concentration of oil initially added increases. In fact, it was found that for dispersions prepared on a reciprocating shaker, as the initial oil concentration increased from 0.1 ml to 20 ml of oil per 500 ml of

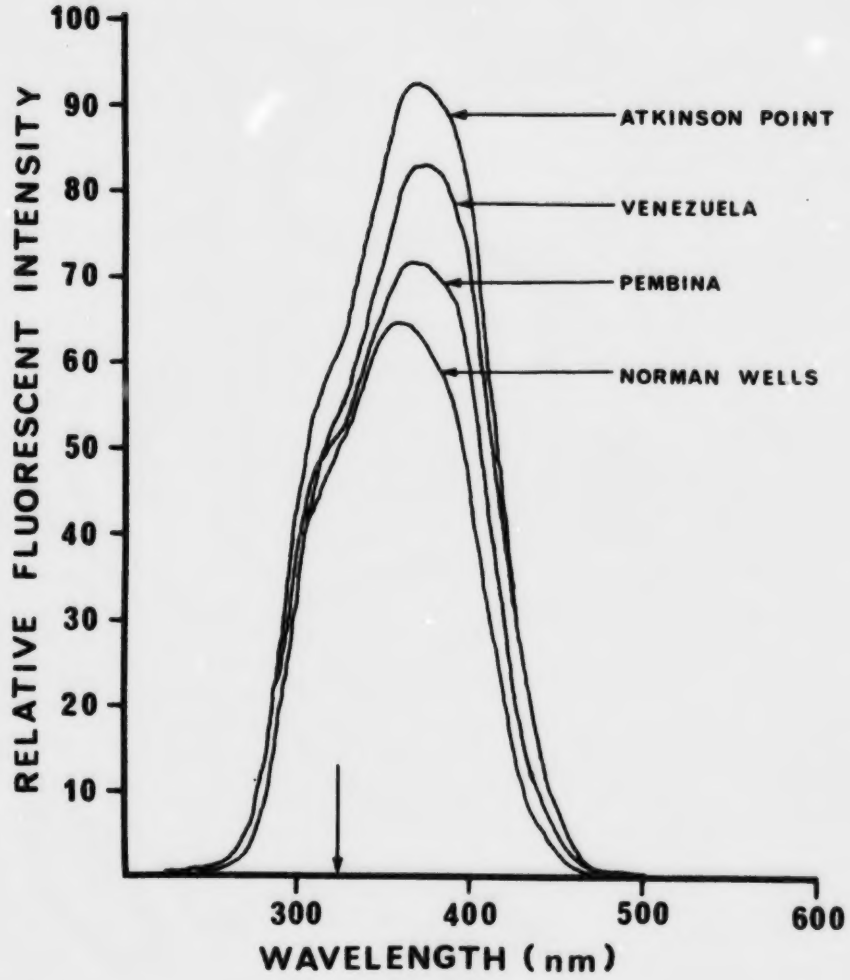

Figure 1. Fluorescence excitation spectra for Norman Wells, Venezuela, Pembina and Atkinson Point crude oils. Emission monochrometer set at 425 nm, slit width 15 nm; 1 µl crude oil dissolved in 10 ml hexane. All scans done at the same instrument setting. Vertical arrow indicates excitation wavelength selected for oil assays.

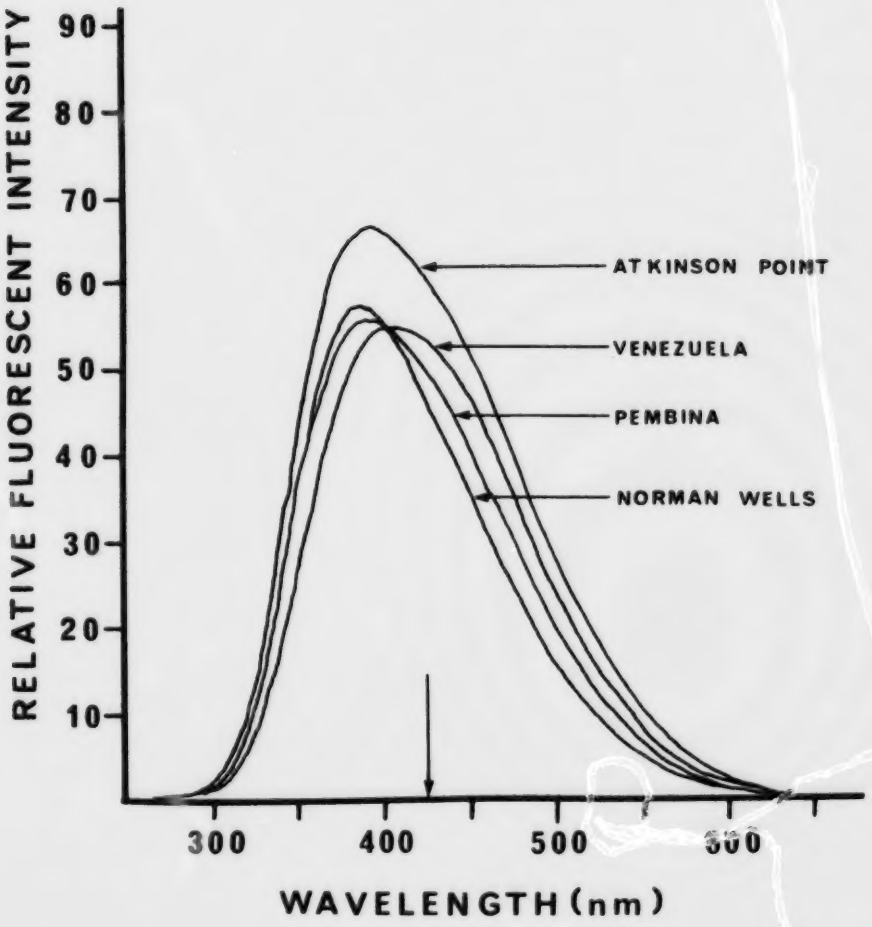

Figure 2. Fluorescence emission spectra for Norman Wells, Venezuela, Pembina and Atkinson Point crude oils. Excitation monochrometer set at 325 nm, slit width 15 nm; 1 µl crude oil dissolved in 10 ml hexane. All scans done at the same instrument setting. Vertical arrow indicates emission wavelength selected for oil assays.

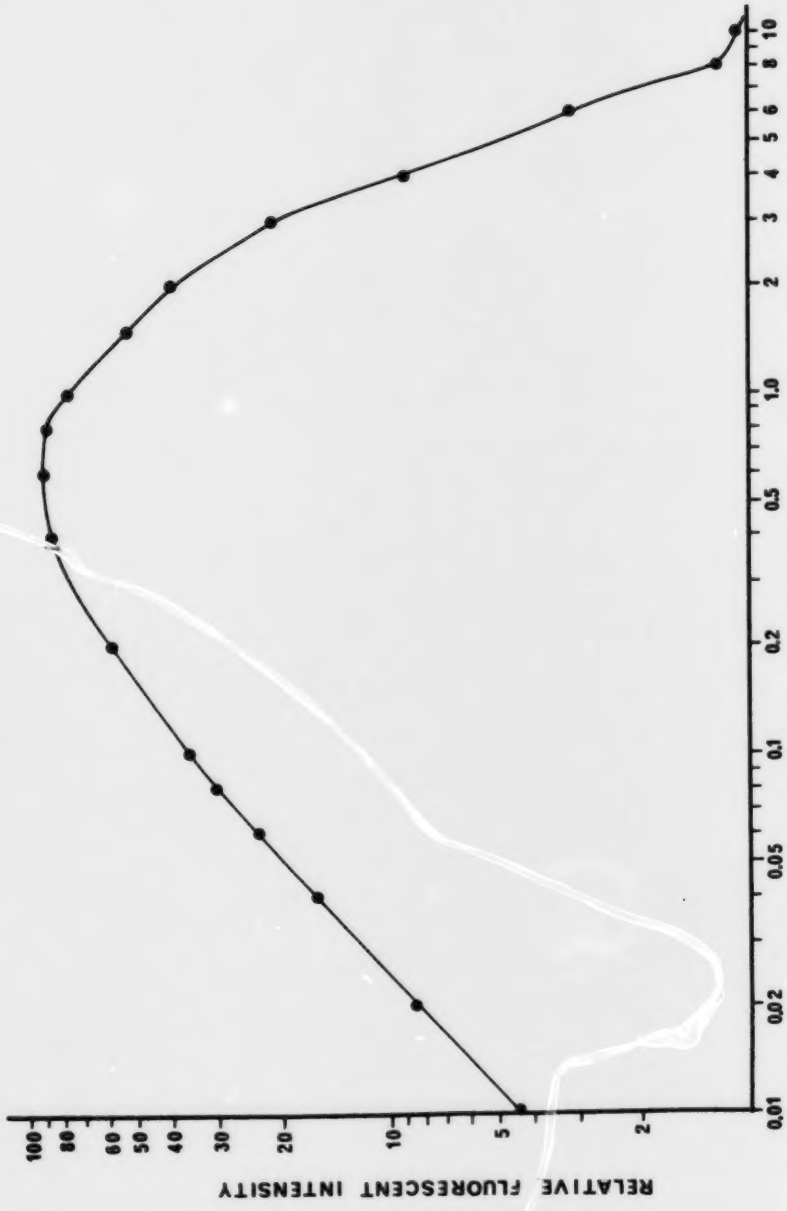

Figure 3. Typical calibration curve for fluorescent assay of crude oil (Pembina crude), illustrating quenching effect at high oil concentrations.

seawater the quantity remaining in the aqueous phase after a standard settling period actually decreased slightly and then remained relatively constant (Figure 4). The dispersion had clearly reached a saturation point, and increasing the initial oil volume above 0.5 ml did not significantly alter the particulate oil content of the resultant semi-stable dispersion. With the preparative techniques employed here, a saturated dispersion of oil was produced with rather low initial oil volumes. For the oils used in this study the saturation point was reached with initial oil additions of between 200 and 400 µl of oil per 500 ml of seawater (Figure 5). At lower concentrations the quantity of oil in the settled dispersions was proportional to the quantity of oil initially added.

In both Figures 4 and 5 there appears to be a slight decline in the quantity of oil dispersed in the aqueous phase as the amount of oil initially added exceeds the saturation level. A similar phenomenon has been observed for dispersions of No. 2 fuel oil. (Anderson et al., 1974). They suggest that as the amount of oil added increases the number of droplets increases with a consequent rise in the rate of coalescence (Smolochowski equation). The resulting dispersion with dissimilar size droplets is less stable than one with fewer similar-sized droplets. "Thus droplet coalescence increases the rate at which oil returns to the surface slick, resulting in lower oil concentrations in the aqueous phase."

5.1.2.2 The influence of mode of agitation

Once the saturation level has been surpassed the particulate oil concentration in a dispersion can only be increased by employing a more violent form of agitation and thus altering the quality of the dispersion. In the present study this was accomplished by using a Waring blender. At low initial concentrations the quantity of oil entrained in the seawater by blending did not differ significantly from that resulting from shaking, because virtually all of the oil was dispersed in a semi-stable form and the rate of coalescence was low (Figure 4). However, with higher initial oil volumes blending resulted in a greater quantity of oil remaining entrained in the water column than did shaking, presumably because much finer oil particles were produced by the former method. Once again, however, it is

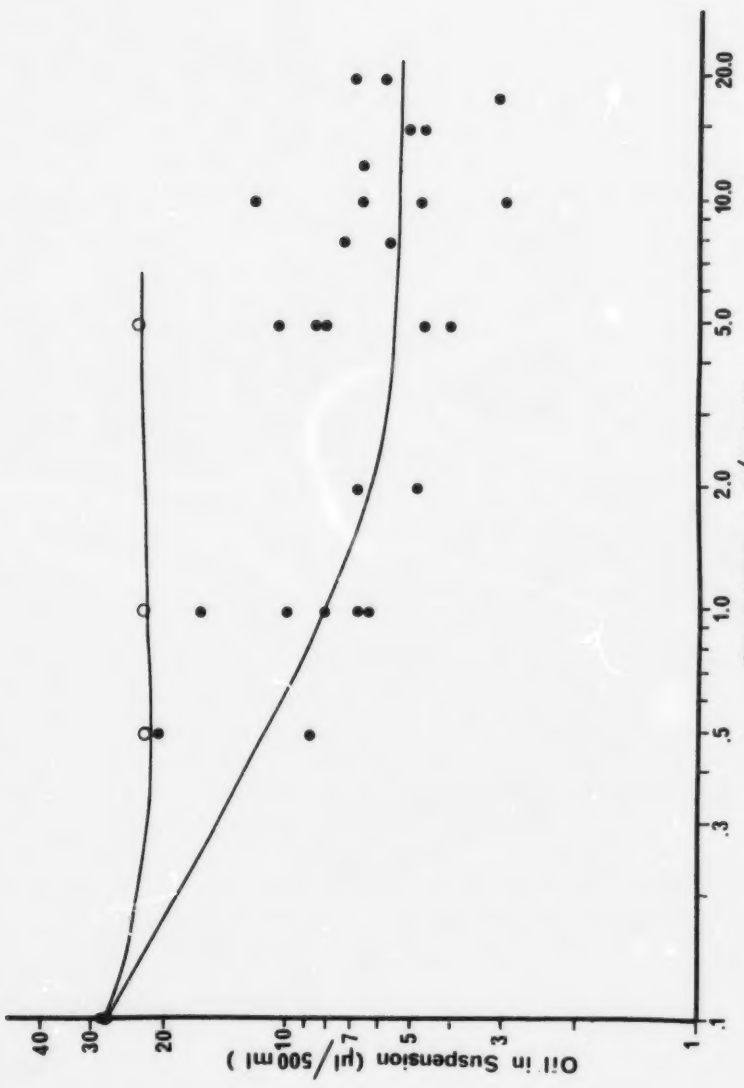

Figure 4. Relationship between the original oil volume (Norman Wells crude) added to the seawater and the concentration remaining in the semi-stable dispersion following standardized preparative procedures. Closed circles: dispersions formed with reciprocating shaker; open circles: dispersions formed with Waring blender.

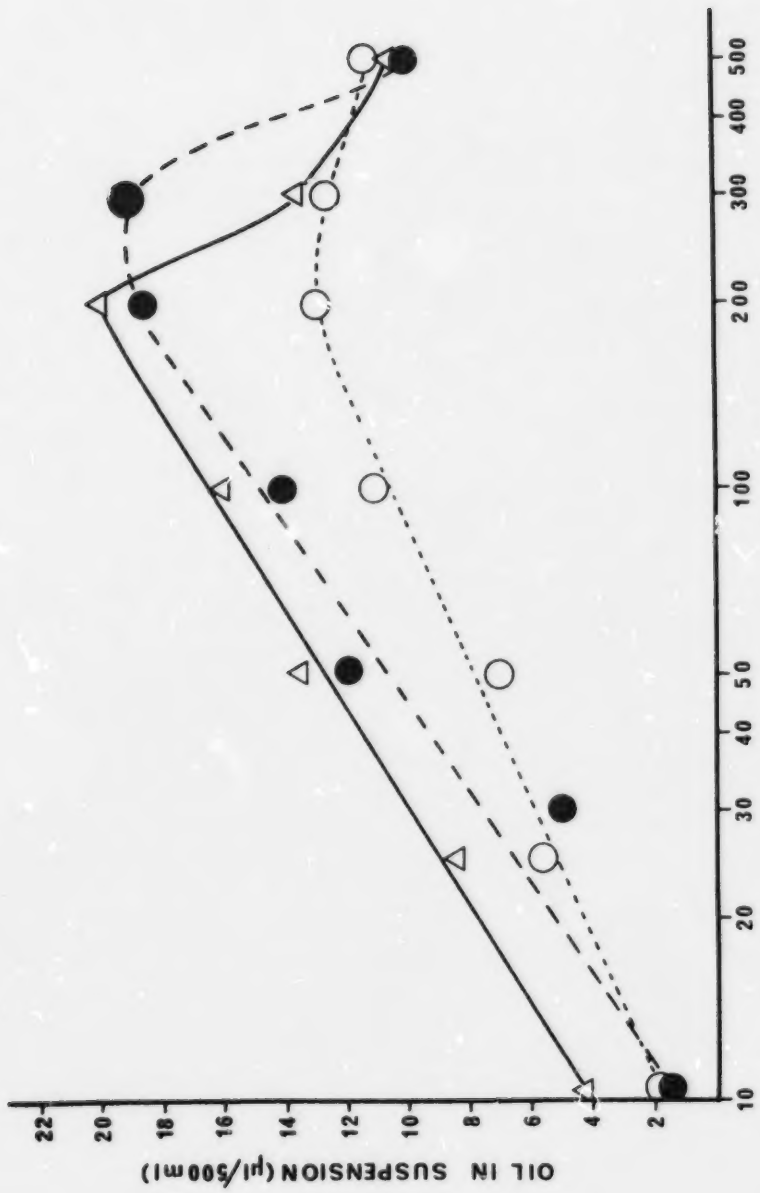

Figure 5. Relationship between the original oil volume added to the seawater and the concentration remaining in the semi-stable dispersion following standardized preparative procedures. All dispersions prepared on a reciprocating shaker with fresh oils: Venezuela crude Δ; Norman Wells crude ●; Atkinson Point crude O.

clear that a saturation point was rapidly reached and increasing the initial volume of oil did not increase the concentration of particulate oil in the aqueous phase beyond a fixed level.

5.1.2.3 The influence of agitation time

Preliminary studies indicated that for oil dispersions prepared on a reciprocating shaker maximal dispersion of the oil in a semi-stable state was achieved within 30 minutes and additional shaking had little further effect. In the case of dispersions prepared with a Waring blender little additional oil appeared to be dispersed in the water by blending for longer than five minutes. Furthermore a prolonged blending period resulted in a significant warming of the seawater that could have increased the rate of loss of volatile components of the oils.

5.1.2.4 The influence of settling time

When crude oil is dispersed in seawater by violent agitation and the mixture then permitted to stand undisturbed, much of the oil rapidly coalesces and separates out as a surface slick. To establish the settling time required to remove most of the rapidly separating fraction of the oil a series of experiments was conducted in which subsurface samples (the lower 450 ml from the separatory funnels) of the water were collected at intervals following dispersion formation and analyzed for oil content. The time courses for loss of oil from the aqueous phase for dispersions of Atkinson Point, Norman Wells and Venezuela crude oils are present in Figures 6, 7 and 8, respectively. The rate of separation of the oil from the seawater was particularly rapid initially as the larger droplets separate out, but gradually declined and eventually reached a virtual plateau. The resulting dispersion was not completely stable; oil was still being lost from the aqueous phase, but at a greatly reduced rate. In this report a settled dispersion of this type that has lost the rapidly coalescing fraction is referred to as a semi-stable dispersion. There appeared to be slight differences in the time required to reach a semi-stable dispersed state with the various types of oil, so the experimental settling times were selected accordingly. Pembina crude was not tested; its physical characteristics are most similar to those of Venezuela crude so comparable settling times were employed for each.

5.1.2.5 Concentrations of dispersed oils during standardized exposure periods

On the basis of the above results three oil volumes were

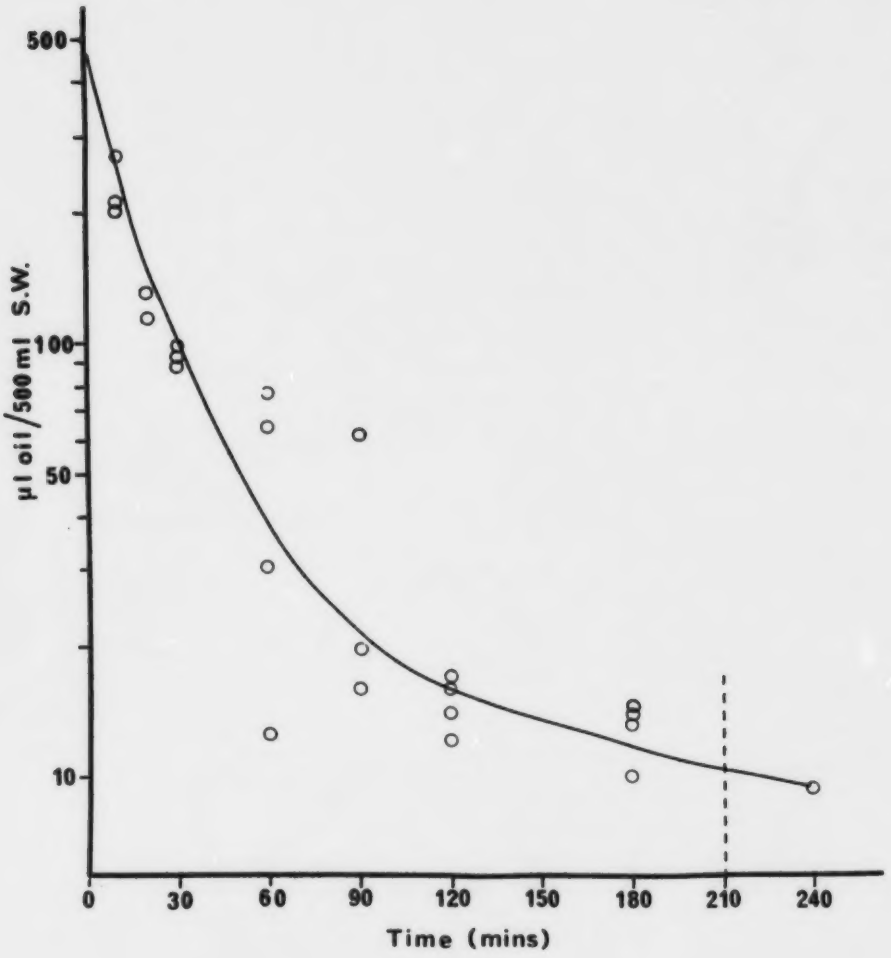

Figure 6. Effect of settling time on the quantity of Atkinson Point crude remaining in a seawater dispersion. Five ml of oil dispersed in 500 ml of seawater on reciprocating shaker. Dashed line indicates settling time adopted for preparation of standard dispersions.

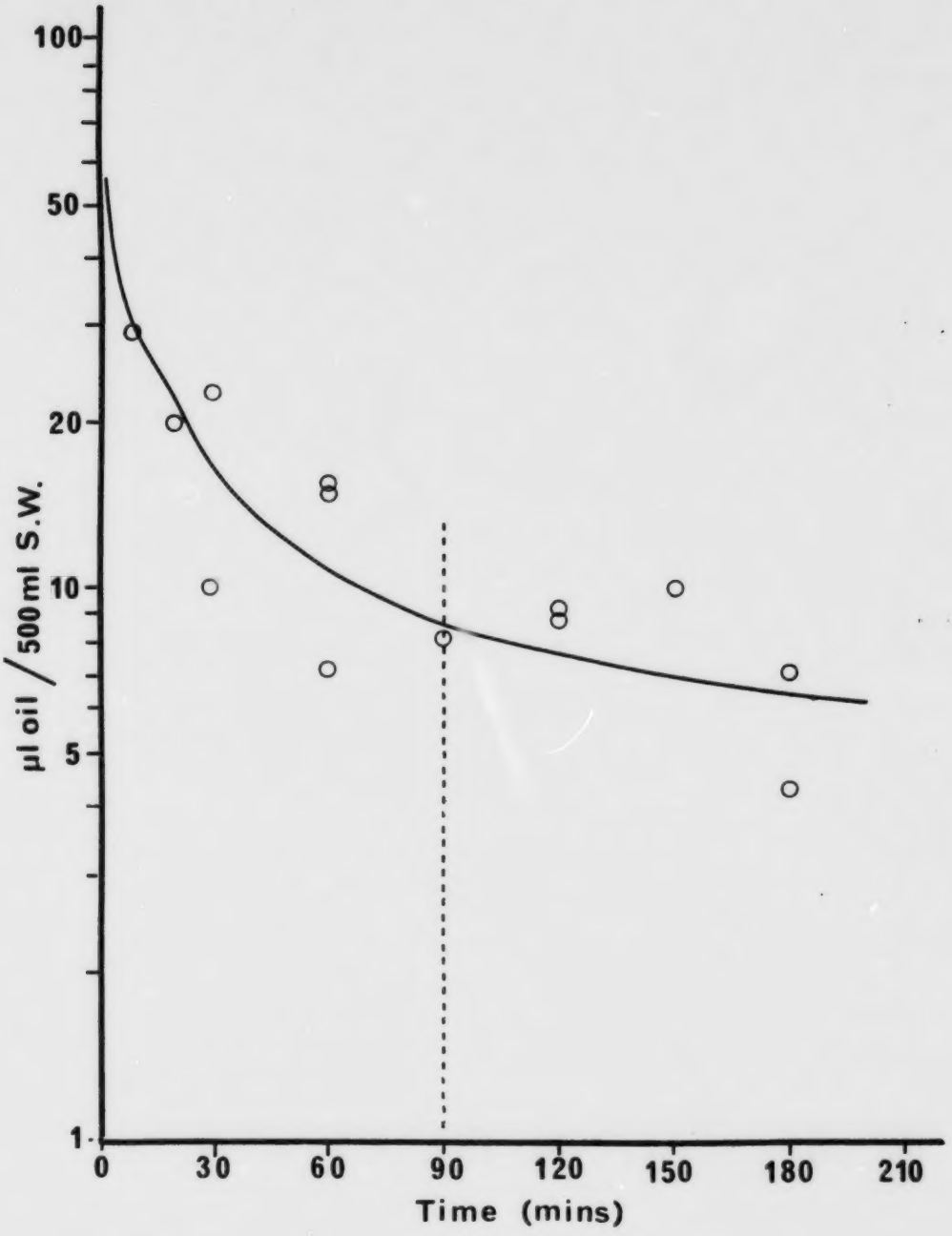

Figure 7. Effect of settling time on the quantity of Norman Wells crude oil remaining in a seawater dispersion. Five ml of oil dispersed in 500 ml of seawater on reciprocating shaker. Dashed line indicates settling time selected for preparation of standard dispersions.

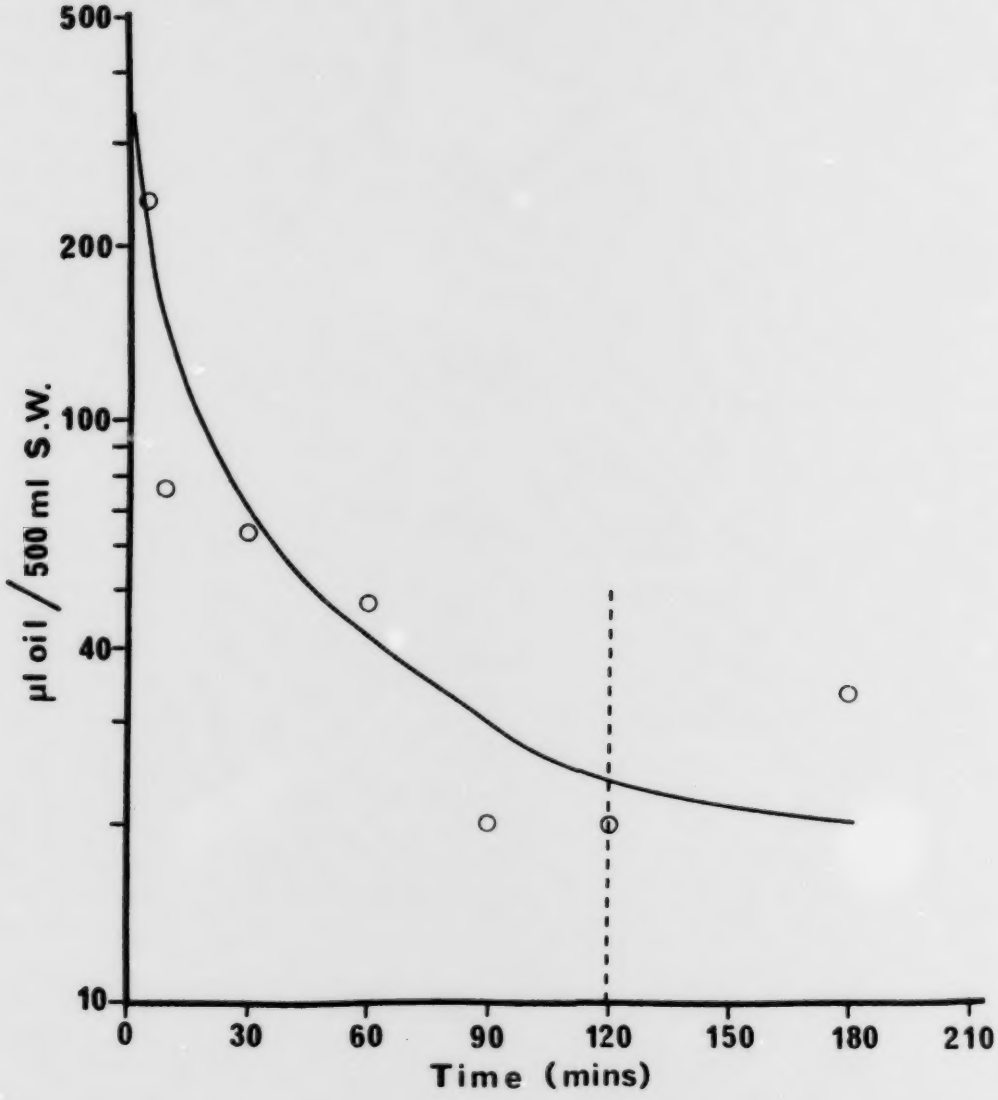

Figure 8. Effect of settling time on the quantity of Venezuela crude oil remaining in a seawater dispersion. Five ml of oil dispersed in 500 ml of seawater on reciprocating shaker. Dashed line indicates settling time adopted for preparation of standard dispersions.

selected for the preparation of the standard emulsions; light emulsion: 25 µl/500 ml of seawater; medium emulsion: 250 µl oil/500 ml of seawater, both prepared on a reciprocating shaker; heavy emulsion: 1000 µl oil/500 ml of seawater prepared in a Waring blender.

The addition of the oil to the seawater on a volume rather than a weight basis was used because of difficulties in weighing to the required degree of accuracy under field laboratory conditions. However, because of the marked differences in viscosity of the oils slight differences in the actual quantities of the oil delivered by pipettes were inevitable. A study was therefore made to determine the variability in the amount of oil delivered by the pipettes and to establish the actual weights of the oils used in the preparation of the standard emulsions. Volumetric samples of the four oils corresponding to those used in emulsion preparation were pipetted into tared weighing vessels and weighed to the nearest 0.001 mg. Five replicate samples for each oil and each volume were measured. Variability of the weights is presented as ½ the range expressed as a percentage of the mean weight. Results are as follows:

a. Venezuela crude;	light (25 µl):	18.847 mg ± 3.0%
	medium (250 µl):	201.762 mg ± 0.7%
	heavy (1000 µl):	809.929 mg ± 0.9%
b. Norman Wells crude;	light (25 µl):	17.623 mg ± 3.8%
	medium (250 µl):	196.733 mg ± 2.2%
	heavy (1000 µl):	797.378 mg ± 2.7%
c. Pembina crude;	light (25 µl):	18.224 mg ± 1.4%
	medium (250 µl):	200.044 mg ± 0.4%
	heavy (1000 µl):	796.146 mg ± 1.3%
d. Atkinson Point crude;	light (25 µl):	20.205 mg ± 1.3%
	medium (250 µl):	214.549 mg ± 2.2%
	heavy (1000 µl):	836.429 mg ± 0.5%

As pointed out in the previous section, a semi-stable dispersion still loses oil but at a greatly reduced rate. Animals placed in such dispersions were thus exposed to a slowly declining concentration of oil. To evaluate the actual range of oil concentrations that the animals were being exposed to during the standard exposure period we measured oil concentration in light, medium and heavy dispersions immediately after settling (corresponding to zero time for animal exposure) and again 24 hours later. Experimental conditions during the 24 hours were identical to those employed for standard animal exposures (section 4.5). Results are presented for Norman Wells, Pembina, Venezuela and Atkinson Point crudes in Figures 9, 10, 11 and in appendix table 1. For routine oil exposures, the dispersions were prepared at room temperature (22° ± 2°C) and animals

exposed to the dispersions at 8° ± 1°C. Under these conditions initial mean concentrations varied slightly for the different oils. Initial oil concentrations for light dispersions generally were in the 10 to 20 ppm range; for medium dispersions in the 20 to 200 ppm range; and for heavy dispersions in the 300 to 1000 ppm range.

Concentrations of oil in the light dispersions were relatively uniform for each of the oils tested, probably because virtually all of the oil remained in the dispersion during the brief settling period. The greater variability in initial oil concentrations of the different oils in medium and heavy concentrations is probably attributable to the fact that these dispersions exceeded the saturation point noted in section 5.1.2.1 and consequently the rate of coalescence during settling assumed greater importance in determining the concentration of oil in the settled dispersions. The markedly different physical properties of the oils probably resulted in different rates of coalescence and different saturation levels.

There was considerable variation among the different oils in the relative quantity of oil lost from the water column during the subsequent 24 hour exposure period. This variation was also presumably attributable largely to differences in physical properties and coalescence behavior of the different oils. Percentage oil losses during the exposure period for the different oils are presented in appendix table 1.

The closed circles in Figures 9, 10 and 11 indicate the geometric mean of the mean initial and final oil concentrations and serve as very crude estimates of the average oil concentrations to which the animals were exposed during the standard 24 hour exposure periods. For Norman Wells crude these values for light, medium and heavy concentrations were approximately 9, 16 and 130 ppm, respectively. Corresponding values for Pembina crude were 20, 30 and 140 ppm; for Venezuela crude, 13, 20 and 250 ppm; and for Atkinson Point crude, 13, 14 and 440 ppm.

In dispersions prepared by the standardized procedures most of the oil appeared to be present in a particulate form. Tests in which the dispersions were filtered through

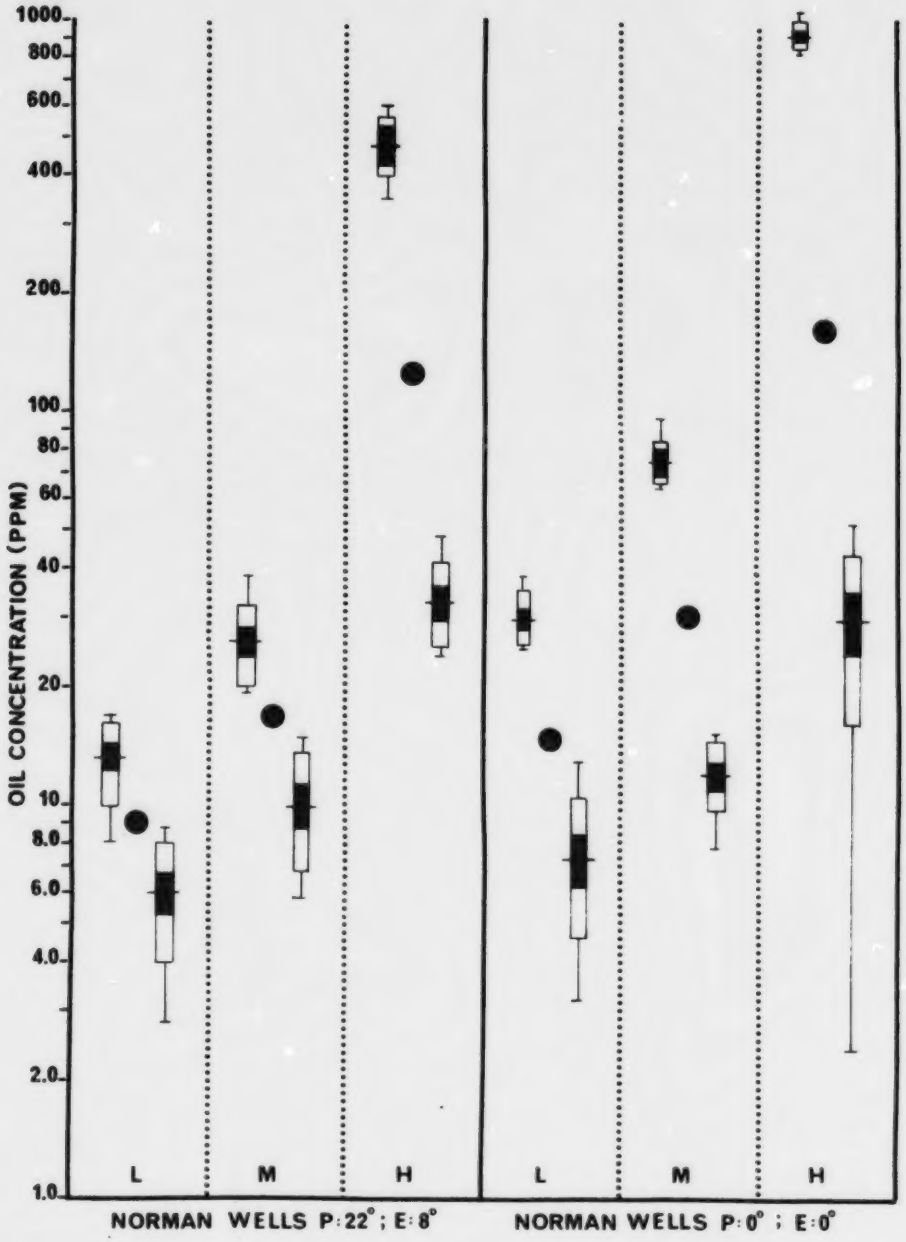

Figure 9. Concentrations of Norman Wells crude oil in seawater dispersions prepared by standard procedure. Initial concentrations (left bar in each column) determined immediately after settling (corresponds to time 0 of exposure period) and final concentration (right bar in each column) determined after standing for 24 hours under standardized bioassay conditions (corresponds to end of exposure period). Light (l), medium (m) and heavy (h) dispersions tested. Preparation temperatures (P) and exposure temperatures (E) indicated. Horizontal line on each bar indicates mean, black portion of bar indicates standard error of the mean, open portion of bar indicates standard deviation and vertical line indicates range of observations. (Appendix table 1).

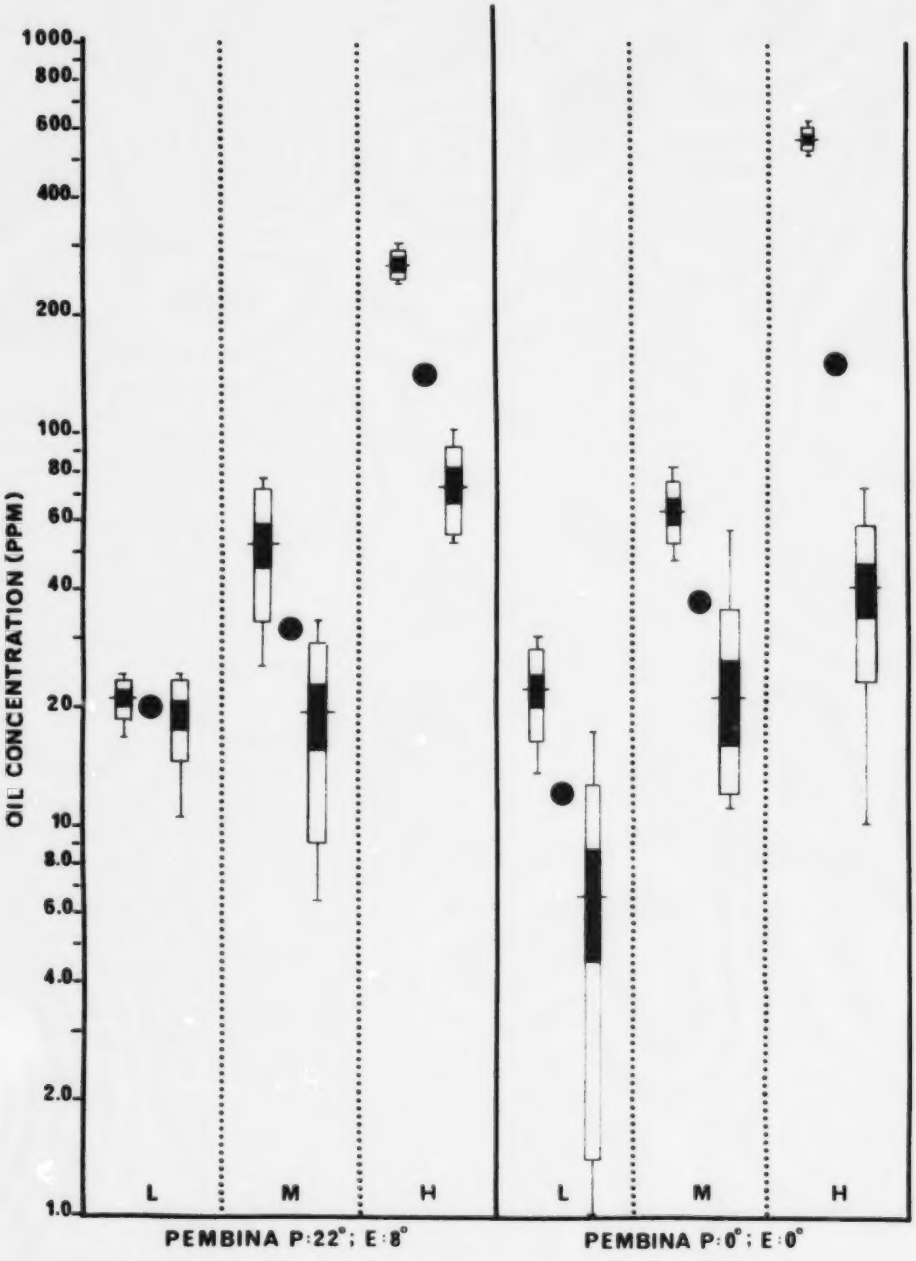

Figure 10. Concentrations of Pembina crude oil in seawater dispersions prepared by standard procedure. See figure 9 for explanation of symbols. (Appendix table 1.)

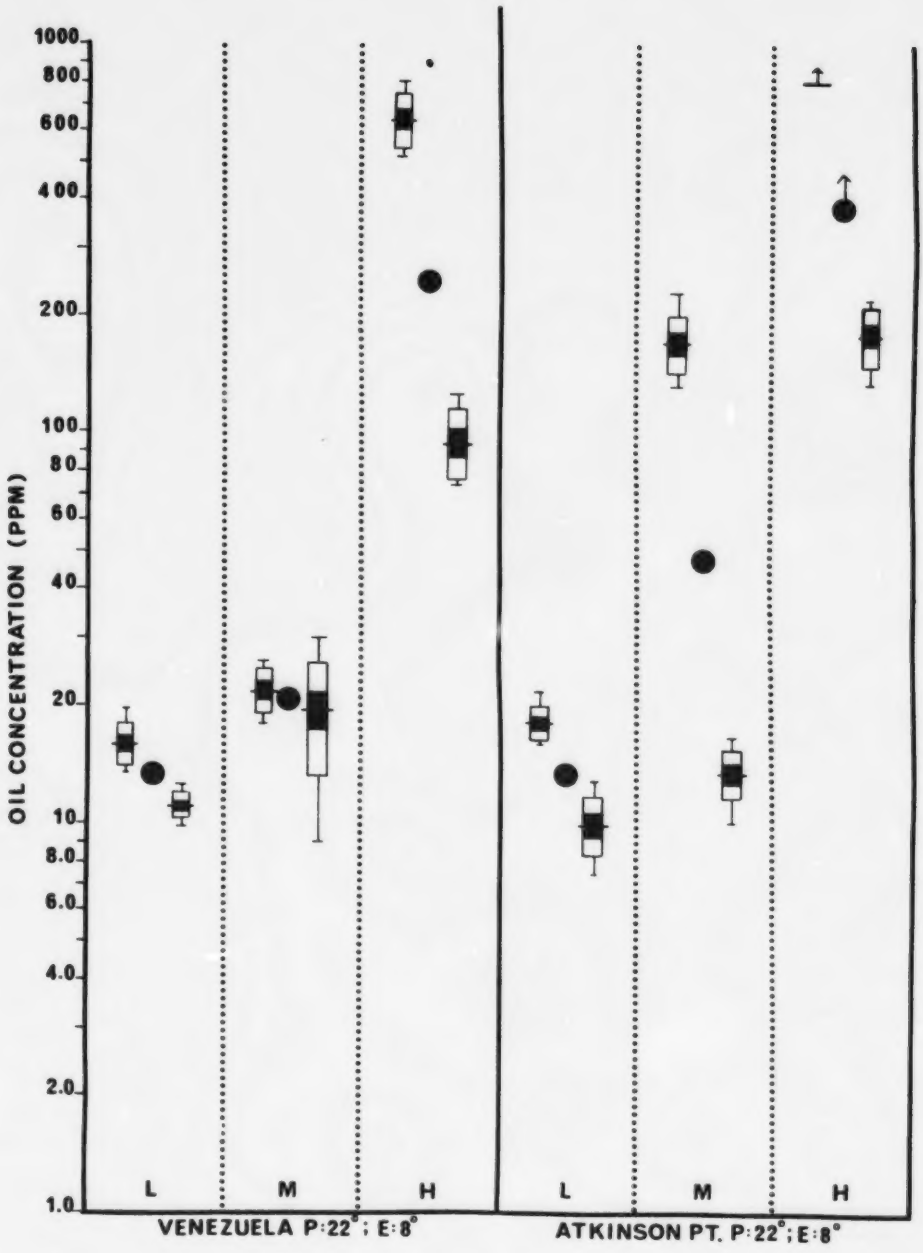

Figure 11. Concentrations of Venezuela and Atkinson Point crude oils in seawater dispersions prepared by standard procedure. See figure 9 for explanation of symbols. (Appendix table 1).

0.45 μ filters revealed that at least 98% of the fluorescent material was retained by the filter. Gordon et al. (1973) similarly reported that in seawater dispersions of various oils 87-98% of the oil occurred in a particulate form.

5.1.2.6 The influence of temperature

The effects of temperature on the formation of seawater dispersions of Norman Wells and Pembina crude oils are illustrated in Figures 9 and 10, respectively. In general the initial concentrations of oils dispersed in the seawater at time zero were significantly greater when the dispersions were prepared and settled at 0°C than at room temperature. With Pembina crude, when light, medium and heavy dispersions were prepared at 0°C the initial oil concentrations were 6%, 23% and 108% higher, respectively than when they were prepared at room temperature. With Norman Wells crude when light, medium and heavy dispersions were prepared at 0°C the initial oil concentrations were 125%, 184% and 90% higher, respectively, than when they were prepared at room temperature. Although in general the concentration of oil initially present in the dispersions was higher at the lower temperature, during the subsequent 24 hour exposure period the loss of oil from the dispersions was greater at 0°C than at 8°C. This is clearly shown in appendix table 1. With Pembina crude the percent loss of oil during the 24 hour exposure period from light, medium and heavy dispersions were 7%, 62% and 73%, respectively at 8°C and 71%, 65% and 92%, respectively at 0°C. With Norman Wells crude the percent losses of oil during the 24 hour exposure period from light, medium and heavy dispersions were 55%, 63% and 93%, respectively at 8°C, and 75%, 84% and 97%, respectively at 0°C. The consequence of this is that although the initial oil concentrations to which the animals were exposed were considerably higher at the lower temperature, the greater loss of oil at the lower temperature during the subsequent 24 hour exposure period means that the average oil concentrations to which the animals were exposed during the 24 hour period (as exemplified by the geometric means in Figures 9 and 10) was only slightly, if at all, higher at the lower temperature. It is clear from the above results that although the general trends, with respect to dispersion formation and temperature were similar for the two oils the magnitudes of the final effects were quite different, presumably a consequence of the markedly different physical characteristics of the oils (section 4.2). Thus the mean concentrations of Pembina crude to which the animals were exposed during the 24 hour period (black dots in Fig. 10) did not differ significantly with exposure temperature. In contrast the mean concentrations of Norman Wells crude to

which the animals were exposed (black dots in Figure 9) were significantly higher during exposure at 0°C than at 8°C.

5.2 Short-term lethal effects of oil dispersions

Standard 96-hour toxicity bioassays were conducted using a variety of invertebrate species common to the Beaufort Sea region and a variety of crude oil types. Detailed results are presented in appendix tables 2 to 13 inclusive.

5.2.1 Relative toxicity of various oils

The relative toxicities of dispersions of Norman Wells, Venezuela, Atkinson Point and Pembina crude oils to *Onisimus affinis*, *Calanus hyperboreas* and *Halitholus cirratus* are shown in Figure 12. The histogram indicates the percentage mortality after 96 hour exposure to heavy dispersions of each of the oils. The number above each bar is the estimated time, in hours, for 50% mortality, derived from a probability plot of the toxicity data. On the basis of these results it is difficult to make generalizations about relative toxicities of the oils that apply to all of the species; each must be examined separately.

Onisimus affinis differs considerably in its tolerance of dispersions of the various oils. No mortalities occurred in light dispersions of any of the oils (appendix table 2). In medium dispersions significant mortalities occurred only with Norman Wells and Venezuela crudes. In both instances mortality did not exceed 10% until at least 48 hours exposure to the dispersions. In heavy dispersions considerable mortality occurred and the toxicity of the oils decreased in the sequence: Venezuela > Norman Wells > Atkinson Point > Pembina. Pembina crude was by far the least toxic, causing only 25% mortality after 96 hours. In contrast virtually 100% mortality occurred over a corresponding period in heavy dispersions of Venezuela and Norman Wells crudes. Mortality occurred most rapidly in Venezuela crude, with 90% of the animals dying within 48 hours Sixty percent died during a corresponding exposure to Norman Wells crude. All of the animals survived a 48 hour exposure to heavy dispersions of Pembina crude.

The planktonic copepod *Calanus hyperboreas* was surprisingly resistant to all of the oils tested. No mortality occurred in heavy dispersions of Atkinson Point and Norman Wells crudes. In none of the oils did the mortality exceed 50% during the 96 hour duration of the test (appendix table 3). Significant mortality occurred only in dispersions of Pembina crude. This result must, however, be viewed with caution, as the animals tested with Pembina crude were from a different collection than those tested with the other three oils, and it is possible that they may have

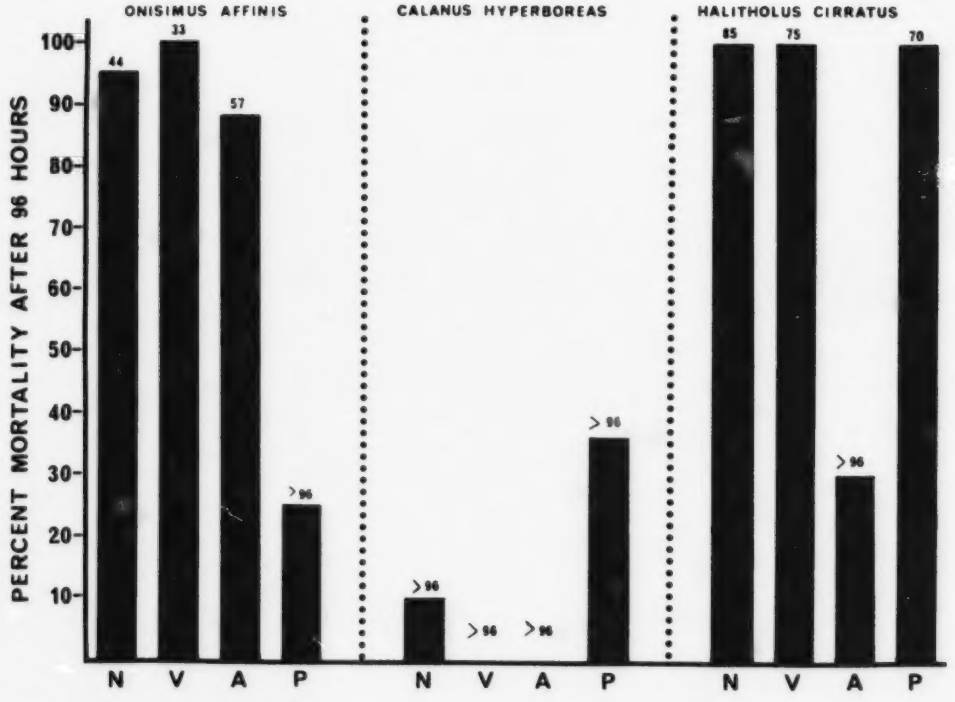

Figure 12. Relative toxicities of Norman Wells (N), Venezuela (V), Atkinson Point (A) and Pembina (P) crude oils to *Onisimus affinis*, *Calanus hyperboreas* and *Halitholus cirratus*. Mortality data for animals exposed to heavy dispersions of the oils. Figures above each bar represent estimated time in hours for 50 percent mortality. *Onisimus* and *Halitholus* exposed at 8°C; *Calanus* exposed at 5°C. (Appendix tables 2,3, and 4).

been subjected to different collecting and handling stresses in spite of the fact that these procedures were standardized as much as possible.

The medusa *Halitholus cirratus* experienced 100% mortality after 96 hours exposure to heavy dispersions of Norman Wells, Venezuela and Pembina crude oils. In contrast in dispersions of Atkinson Point crude only 30% mortality occurred during a comparable exposure period. Little or no mortality occurred in light and medium dispersions of any of the oils (appendix table 4). Death occurred most rapidly in Pembina and Venezuela crudes, although in all cases at least 50% of the animals survived 48 hours exposure to heavy dispersions. It should be remembered that the criterion for death used in these tests was the total absence of contractions of the bell even when stimulated lightly. Reference should be made to the section dealing with the effects of oil on activity to properly appreciate the impact of oil dispersions on this species. In general toxicity appears to decrease in the sequence: Pembina > Venezuela > Norman Wells > Atkinson Point.

5.2.2 Relative species sensitivity

Species differed markedly in their sensitivity to seawater dispersions of Norman Wells crude oil (Figure 13). This histogram is only a rough guide to relative sensitivity, as it is based upon percent mortality following 96 hours exposure to heavy dispersions of the oil. The animals, however, clearly fall into two distinct groups, those that were particularly sensitive and those that were particularly resistant to short-term exposure to dispersed oil. The detailed data for each of the species are presented in appendix table 5.

Of all the species tested the fry of the sculpin *Myoxocephalus quadricornis* appeared to be the most sensitive to oil. All of the animals died within 24 hours of exposure to heavy dispersions. On the basis of total mortality after 96 hours the medusa *Halitholus cirratus* appeared to be next most sensitive; however, other species exhibited greater mortalities over shorter time periods and at lower concentrations.

The benthic amphipods *Onisimus affinis* and *Corophium clarencense* were very sensitive, particularly in view of the fact that these were the only species that experienced significant mortalities in medium dispersions.

Barnacles, *Balanus crenatus*, are considered very sensitive, although the criterion for mortality is less satisfactory than that for other species because of the animal's ability to isolate itself from the environment by tightly closing its opercular plates. Individuals were considered dead when the opercular plates

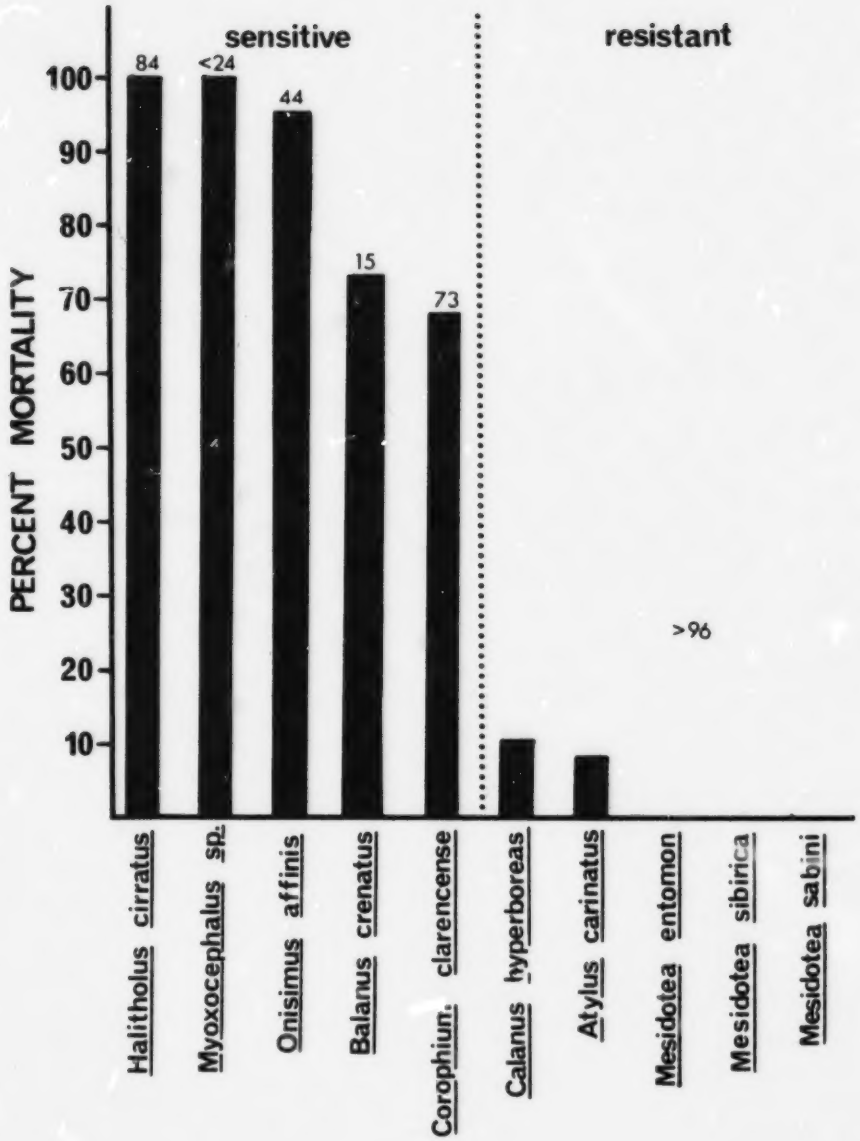

Figure 13. Relative toxicity of heavy dispersions of Norman Wells crude oil to various arctic marine species. Percent mortality determined after 96 hours exposure under standard conditions. Figures above each bar represent estimated time in hours for 50 percent mortality. (Appendix table 5).

gaped open and did not close in response to light stimulation with a probe. Only animals exposed to heavy dispersions exhibited this behavior during the course of the test.

The copepod *Calanus hyperboreas*, the amphipod *Atylus carinatus* and the three isopod species *Mesidotea entomon*, *M. sibirica* and *M. sabini* appeared to be extremely resistant to crude oil dispersions. Little or no mortality was observed even after 96 hours exposure to the heavy dispersion.

The data for the benthic cumacean *Brachydiastylis resima* is inconclusive and is not included in Figure 14. The considerable mortality among control animals was almost certainly attributable to unavoidable damage to these rather delicate animals sustained during collection and sorting of bottom samples. The fact that the maximum mortality observed for controls during the test was 45%, while that among animals exposed to heavy emulsions for 96 hours was only 50% suggests that this species may in fact be rather resistant to crude oil dispersions.

5.2.3 Influence of dispersants on toxicity

Short-term bioassays were conducted to examine the effect of dispersing the crude oil with the emulsifier Corexit 8660 on the toxicity of the oil to the amphipod *Onisimus affinis*. A summary of the results of these tests is presented in Figure 14. The Corexit alone was essentially non-toxic under the test conditions; even at the highest concentration tested mortality did not exceed 10%. In contrast, the oil/dispersant mixtures although causing low mortality in light and medium dispersions killed almost all of the animals exposed to heavy dispersions A comparison of the relative toxicities of oil alone and in combination with Corexit is presented in Figure 15. The dispersant appeared to have little effect on the toxicity of Norman Wells, Venezuela and Atkinson Point crudes. In the case of Pembina crude mortality in heavy dispersions was greatly increased when Corexit was present. The general lack of effect of Corexit is possibly attributable to the fact that in the experimental procedure employed the oil was already in a highly dispersed form. Corexit, although it would undoubtedly facilitate the formation of the dispersions and perhaps stabilize them, it may not significantly increase the quantity of oil in the dispersion to which the animals are exposed. The explanation for the increased toxicity of Pembina crude in the presence of Corexit is uncertain in the absence of data on the influence of the dispersant on accommodation of dispersed oils in seawater.

5.2.4 Influence of suspended sediment on toxicity

Routine toxicity bioassays were carried out with oil

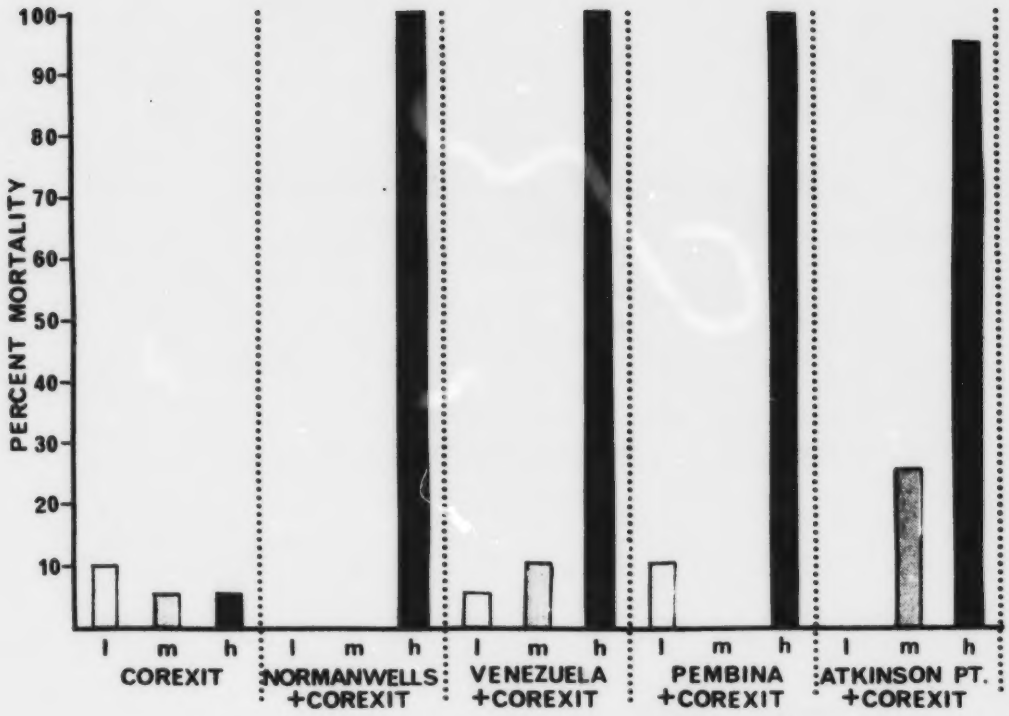

Figure 14. Relative toxicity to *Onisimus affinis* of the dispersant Corexit alone and in combination with various quantities of Norman Wells, Venezuela, Pembina and Atkinson Point crude oils. Standard light (l), medium (m) and heavy (h) dispersions tested at 8°C. Percent mortality determined after 96 hours exposure. (Appendix table 6).

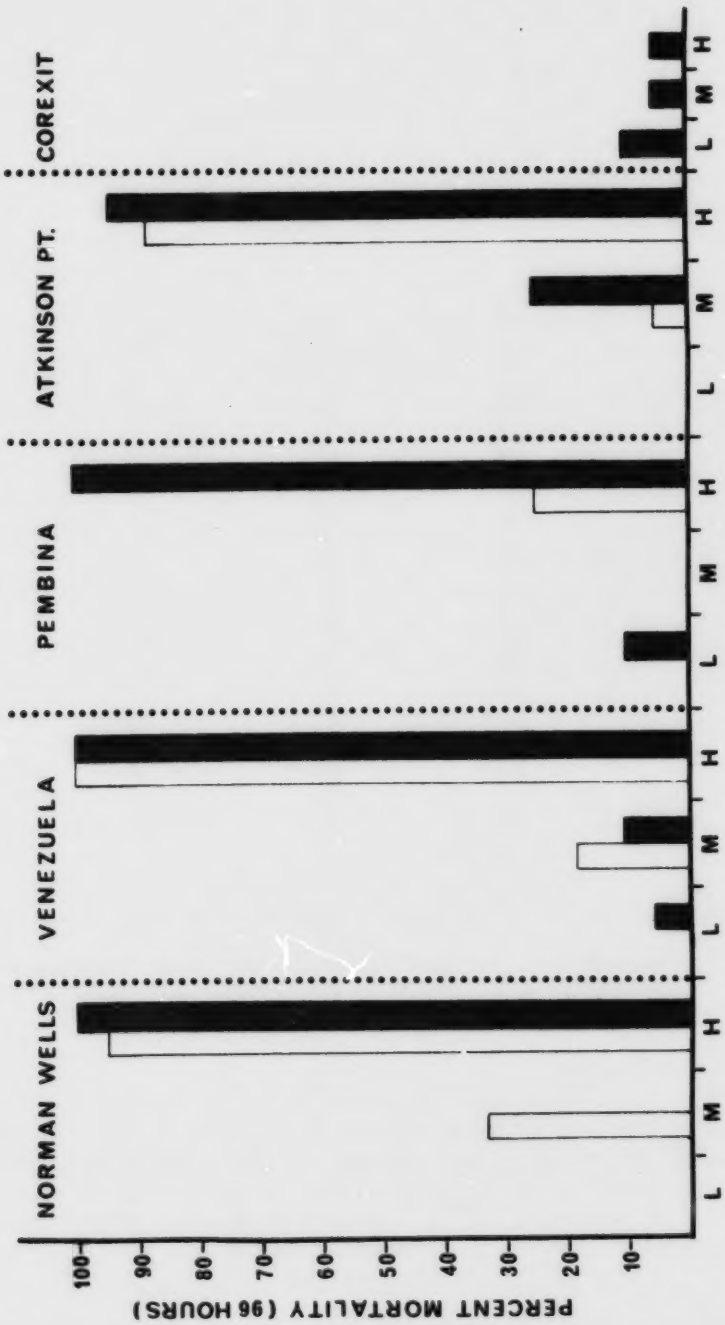

Figure 15. Comparison of toxicity to *Onisimus affinis* of various crude oils along (white bars) and in combination (1:1) with Corexit dispersant (black bars). (Appendix table 7).

dispersions prepared in seawater filtered to remove all natural particulate matter greater than 1μ. In view of the variably turbid nature of the inshore Beaufort Sea water a preliminary test was carried out to determine the effect of sediment in the water column on the toxicity of seawater dispersions of Norman Wells and Pembina crude oils to the amphipod *Onisimus affinis* (Figure 16). The presence of sediment appeared to have little effect on the toxicity of Norman Wells crude. A possible explanation for the generally low toxicity levels observed in this test with Norman Wells crude compared to levels observed in earlier tests with the same oil is discussed in section 5.2.5. In contrast the presence of sediment in the water column appeared to enhance the toxicity of Pembina crude considerably. The explanation for this enhancement is uncertain and the whole question of oil/sediment interactions needs to be looked at much more closely.

5.2.5 Influence of season on toxicity

Examination of the short-term toxicity data for *Onisimus affinis* revealed a possible seasonal variability in sensitivity to Norman Wells crude oil (Figure 17). The animals appeared to be much more sensitive to the oil dispersions in the summer (July) than in winter and early spring (March and May), even though the experimental conditions during the tests were similar. However, at present we cannot rule out the possibility that factors other than strictly seasonal ones were responsible for the observed effect. The relationship was noted after the fact, and so must be considered tentative until studies designed to look specifically at seasonal variations in sensitivity are completed. In this connection, it is pertinent to note that Crapp (1971) reported that certain intertidal species exhibited comparable seasonal variations in sensitivity to oil dispersants. The molluscs *Nucella lapillus* and *Gibbula umbilicalis* were much more sensitive to the toxicant in summer than in winter. In contrast to the results observed with Norman Wells crude, the sensitivity of *Onisimus* to Pembina crude did not appear to differ significantly in summer and winter (appendix table 11). In both seasons the mortalities remained uniformly low.

5.2.6 Influence of temperature on toxicity

The effects of temperature on the toxicities of Pembina crude and Norman Wells crude to *Onisimus affinis* are illustrated in Figures 18 and 19. Dispersions were prepared at either room temperature (22°C) or at 0°C and groups of animals were exposed to these dispersions at either 0°C or 8°C.

Animals exposed to dispersions of Pembina crude at 0°C were much more tolerant of the oil than those exposed at 8°C. The temperature of preparation appeared to have little effect on the toxicity of the dispersions; mortality in dispersions

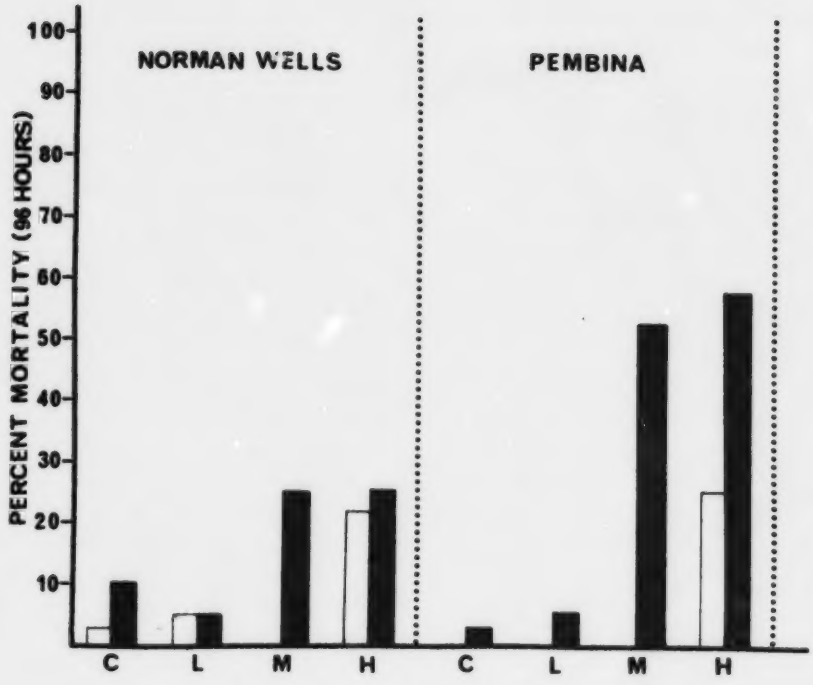

Figure 16. Effect of suspended sediment on the toxicity of dispersions of Norman Wells and Pembina crude oils to *Onisimus affinis*. White bars: without suspended sediment; black bars: with suspended sediment. (Appendix tables 8 and 9).

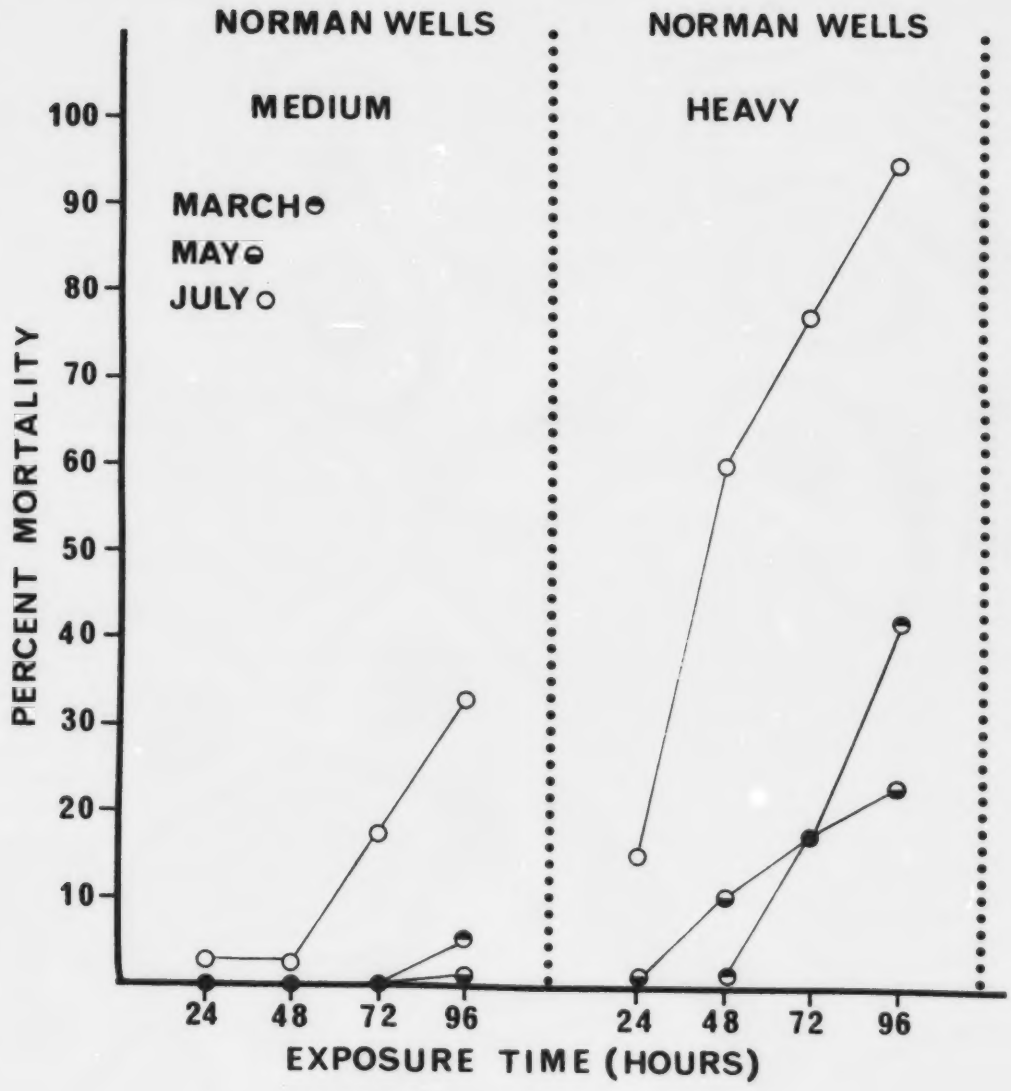

Figure 17. Seasonal variation in sensitivity of *Osisimus affinis* to medium and heavy dispersion of Norman Wells crude oil. Animals exposed to oil at 8°C in all cases. Appendix table 10).

prepared at 22°C and exposed at 8°C was similar to that in dispersions prepared at 0°C and exposed at 8°C. This is not surprisingly in view of the earlier observation (Figure 10) that the mean concentration of Pembina crude to which the animals were exposed during the 24 hour exposure period did not appear to vary greatly with temperature.

In the case of exposure of *Onisimus* to dispersions of Norman Wells crude temperature appeared to play two opposing roles in influencing the degree of toxicity (Figure 19). At lower temperatures the animals were less sensitive to the toxic effects of oil dispersions. Thus for dispersions prepared at room temperature, the mortality of animals exposed at 8°C was considerably greater than that of ones exposed at 0°C. Similarly, for dispersions prepared at 0°C, the mortality of animals exposed at 8°C was greater than that of animals exposed at 0°C. These are essentially similar to the results obtained with Pembina crude.

However, this tendency on the part of the animals to be more tolerant of toxicants at low temperatures appeared to be offset to some extent by an increased toxicity of the dispersions prepared at lower temperatures. Oil dispersions prepared at 0°C were more toxic to *Onisimus* than were dispersions prepared at room temperature. This trend was evident for animals exposed to the dispersions at both 0°C and 8°C (Figure 19). This effect is probably largely attributable to the fact that animals placed in dispersions prepared at the lower temperature were exposed to a slightly greater mean concentration of dispersed oil during the 24 hours test period (Figure 9). It is also likely that greater quantities of toxic volatile components of the oil were lost during preparation at the higher temperatures.

Additional toxicity tests were conducted using a sample of Norman Wells crude oil that had been trapped for several months under, and within, the annual ice of a small bay on Cape Parry, N.W.T. The oil had been released under the ice in November, 1974 and samples were recovered in May, 1975. For a more complete description of this controlled oil spill refer to Beaufort Sea Project Technical Report #27. Standard dispersions were prepared from this sub-ice oil at 0°C and groups of *Onisimus* were exposed for 96 hours at 0°C under standardized bioassay conditions. The mortality of animals exposed to dispersions of the sub-ice oil was not significantly different from that observed with dispersions of fresh crude oil under comparable experimental conditions (Figure 19). It is clear that the toxicity of oil trapped within sea ice does not diminish significantly during the course of several months.

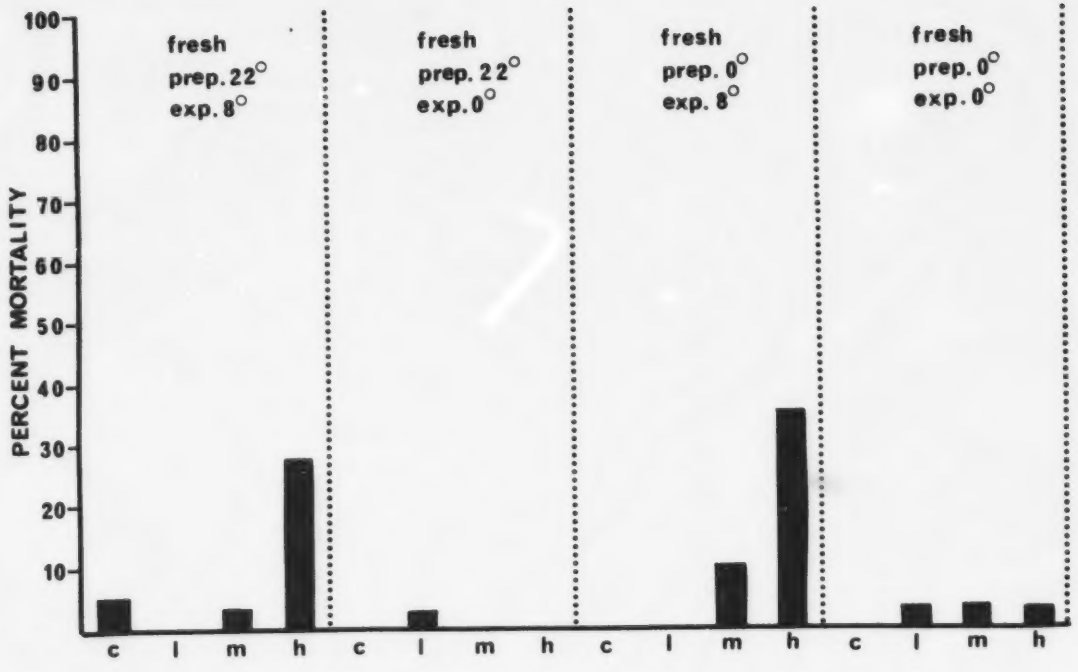

Figure 18. Effect of preparation and exposure temperatures on the toxicities of light (l), medium (m) and heavy (h) dispersions of fresh Pembina crude oil to *Onisimus affinis*. Control (c) mortalities indicated. Percent mortality determined after 96 hours exposure. (Appendix table 12).

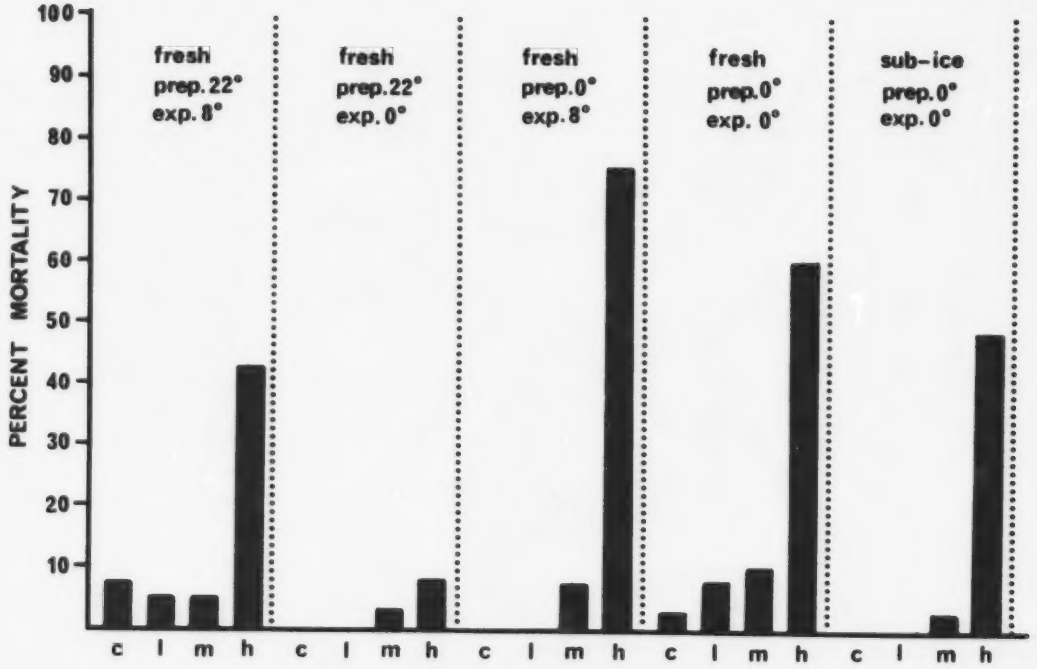

Figure 19. Effect of preparation and exposure temperatures on the toxicities of light (l), medium (m) and heavy (h) dispersions of fresh and sub-ice weathered (see text for details) Norman Wells crude oil to *Onisimus affinis*. Control (c) mortalities indicated. Percent mortality determined after 96 hours exposure (Appendix table 13).

5.3 Relative toxicity of oil contaminated sediments

To examine the toxicity of oil-contaminated sediments to benthic organisms, groups of *Onisimus* were exposed at intervals to small quantities of bottom sediments experimentally contaminated (section 4.6.2) with various quantities of fresh Norman Well, Pembina and Atkinson Point crude oils. Results for experiments conducted at 0°C and 10°C are presented in Figures 20 and 21, respectively.

Mortalities in medium contaminated sediments (0.25 ml of oil/10 gm dry sediment) were generally low at both 0°C and 10°C during all of the exposure periods. The highest mortality at this oil level occurred during the first exposure period at 0°C with Norman Wells crude, but by the second exposure period mortality was minimal.

Very high mortalities occurred when the animals were exposed to sediment contaminated with heavy concentrations (1.0 ml of oil/10 gm dry sediment) of all three oils at both 0°C and 10°C. It is clear that under the experimental conditions employed, the LC_{50} concentration (the quantity of oil in the sediment that results in death of 50% of the exposed animals within 10 days) is reached when sediment is agitated with between 0.25 ml and 1.0 ml of oil per 10 grams of dry sediment. The actual quantity of oil adsorbed onto the sediment was considerably less than the original volume added to the system, because much of the oil coalesced as a surface slick and was discarded during the initial settling of the contaminated sediment. We have not yet undertaken a quantitative fluorescence assay of the actual quantities of oil adsorbed on the sediments following our standardized contamination procedure.

However, the results do illustrate several features related to the relative toxicities of different oils when entrained in sediments. The three oils tested show markedly different toxicity/time relationships. All three oils caused high mortalities during the initial exposure period at both 0°C and 10°C, although at 0°C the toxicity of Atkinson Point crude was considerably less than that of the other two oils. The toxicities of all three oils gradually declined during succeeding exposure periods at both 0°C and 10°C. At both temperatures the toxicity of Atkinson Point contaminated sediment declined the most rapidly, with the greatest decline occurring between the first and second exposure periods. In contrast, Norman Wells contaminated sediment retained a much higher degree of toxicity for a longer period of time than did sediment contaminated with either of the other two oils. Even after 50-60 days at either 0°C or 10°C exposure to the contaminated sediment still resulted in more than 50% mortality among *Onisimus* exposed for a 10 day period. Pembina crude contaminated sediment was initially just as toxic as that contaminated with Norman Wells crude.

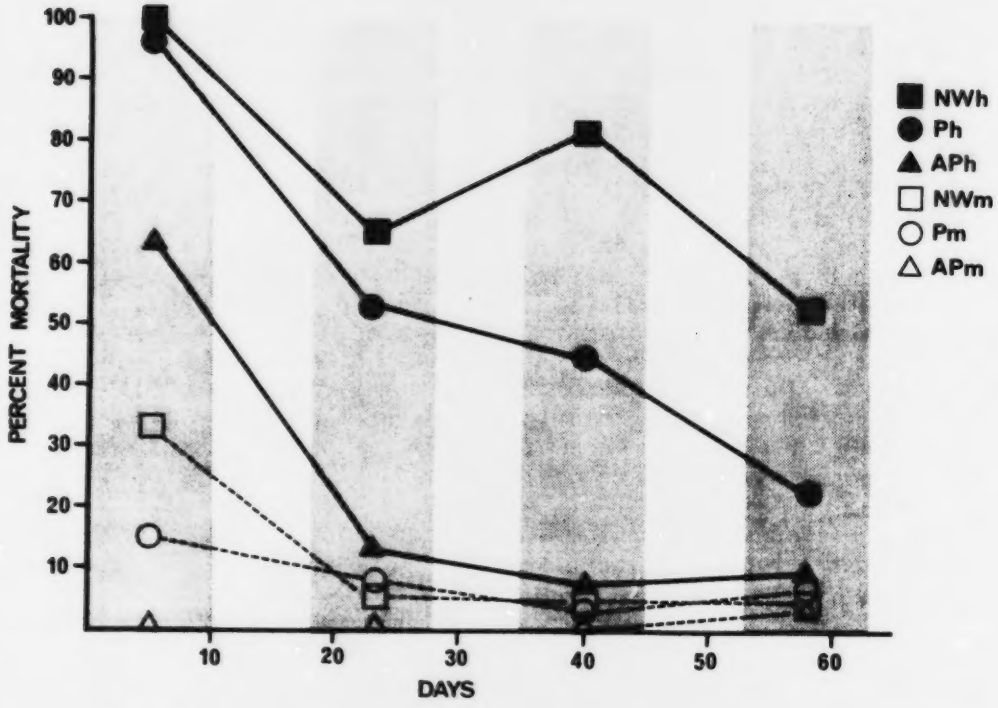

Figure 20. Relative toxicities to *Onisimus affinis* over a period of time of sediments contaminated with medium (m:0.25 ml oil/10 gm dry sediment) and heavy (h:1.0 ml oil/10 gm dry sediment) concentrations of Norman Wells, Pembina and Atkinson Point crude oils. Test conducted at 0°C. Percent mortality during each 10 day exposure (shaded area) indicated at mid point of exposure period. Each exposure group consisted of 20 animals. Fresh groups of animals exposed to sediment at beginning of each exposure period. (Appendix table 14).

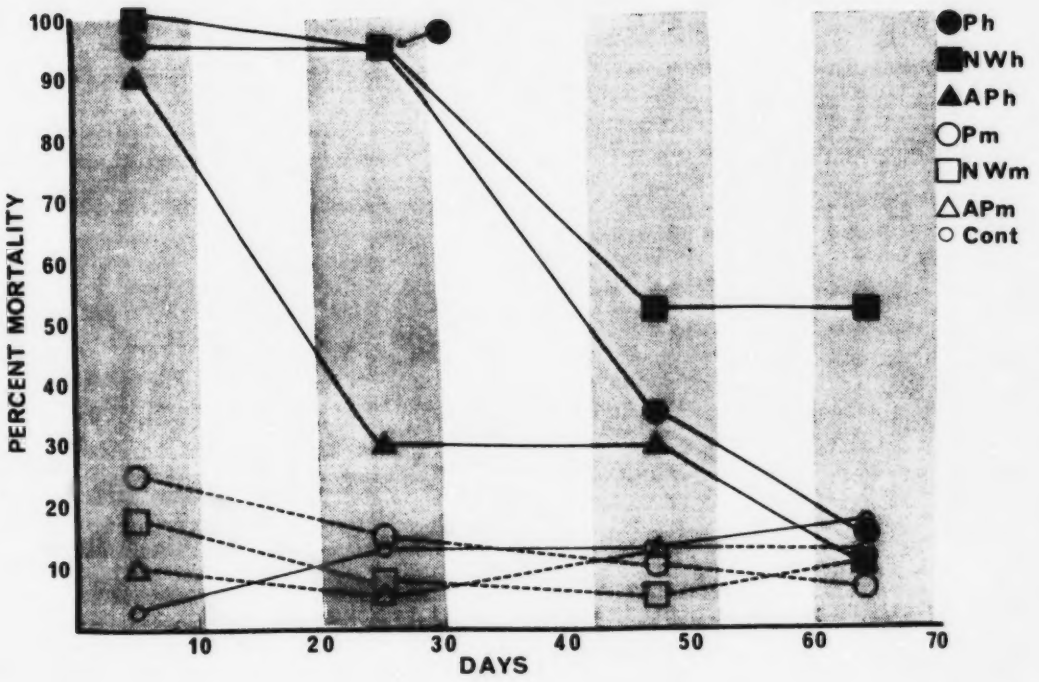

Figure 21. Relative toxicities to *Onisimus affinis* over a period of time of sediments contaminated with Norman Wells, Pembina and Atkinson Point crude oils. Tests conducted at 10°C. Other experimental conditions as in Figure 20. (Appendix table 15).

However, Pembina was intermediate between Atkinson Point and Norman Wells in the rate of diminution of toxicity with time. At both 0°C and 10°C toxicity declined uniformly with time so that after 50-60 days mortality was less than 30%.

There appears to be no consistent effect of temperature upon the rate of loss of toxicity by the oil contaminated sediments. This aspect of the problem appears to be complicated by the same opposing tendencies, with respect to temperature, observed in section 5.2.6, namely, that although the animals are more susceptible to the toxicants at higher temperatures, the toxicants themselves are dissipated by weathering more rapidly at the higher temperature.

5.4 Sub-lethal physiological effects of exposure to oil dispersions

Studies were carried out to investigate the effect of 24 hour exposure to sublethal dispersions of a variety of northern crude oils on the physiological functioning of several arctic marine invertebrates. Respiratory metabolism and locomotory activity were employed as sensitive indicators of physiological state.

5.4.1 Effects on respiratory metabolism

5.4.1.1 Effect of exposure to dispersion of crude oil alone

Following 24 hours exposure to dispersions of several northern crude oils the metabolic rate of *Onisimus affinis* exhibited what at first glance appeared to be a disconcerting variability in response (Figure 22). The treatment appeared to increase, decrease or have little significant effect on metabolic rate, depending upon the oil type and concentration. However, closer examination revealed a general trend recurring consistently within each oil grouping. Exposure to the lowest dispersion concentrations tested (light), without exception, resulted in a depression of metabolism ranging from 7% to 22%. Exposure to medium dispersions of Atkinson Point, Venezuela and Pembina crudes resulted in a slightly greater inhibition of metabolism, ranging from 13% to 25%. In contrast the metabolic rate of animals exposed to medium dispersions of Norman Wells crude was not significantly different from that of controls. Exposure to heavy dispersions of Norman Wells, Venezuela and Pembina crudes resulted in significant increases in metabolic rate ranging from 16% to 41%. In contrast the metabolic rate of animals exposed to heavy dispersions of Atkinson Point crude was not significantly different from that of controls.

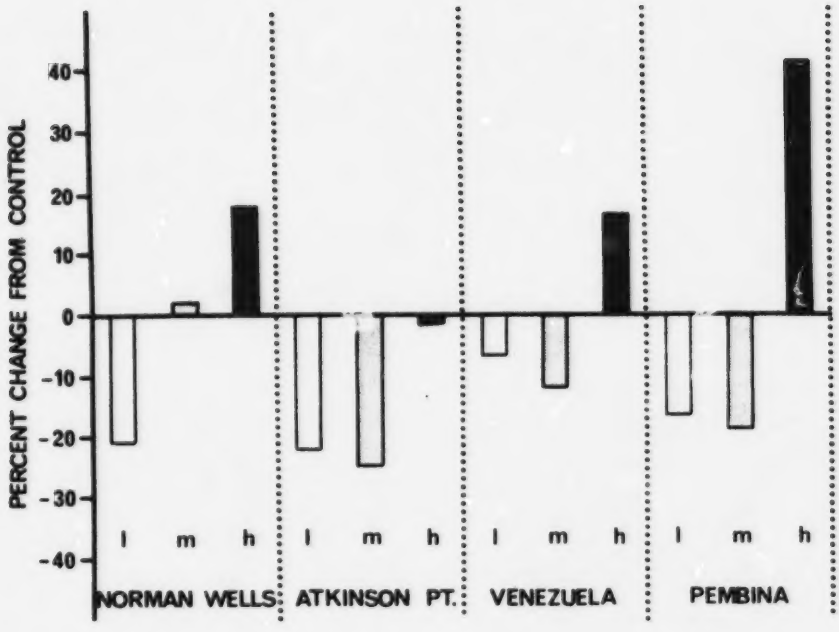

Figure 22. Effect of 24 hour exposure (at 8°C) to light, medium and heavy dispersions of Norman Wells, Atkinson Point Venezuela and Pembina crude oils on the respiratory metabolism (at 8°C) of the amphipod *Onisimus affinis*. (Appendix table 16).

The important point to note is that in each case there was an initial inhibition of metabolism at low oil concentrations followed by a reversal of the inhibition as the oil concentration increased. This reversal in some cases resulted in an actual enhancement of metabolic rate relative to that of the controls. The reversal occurred consistently with each of the oils tested, although the concentration at which the reversal took place and the magnitude of the depression and subsequent enhancement differed somewhat for the different oil types. In view of the considerable differences in physical and chemical characteristics of the different oils this variability in response was not wholly unexpected.

There were a number of oil specific differences in metabolic response that may be relevant in the discussion of possible explanations for the observed effects (see section 6.2.2). The reversal of inhibition was initiated at a lower dispersion concentration with Norman Wells crude than with any of the other oils. No enhancement of metabolism occurred following exposure to Atkinson Point crude, even at the highest concentration tested. In general the magnitude of the initial inhibition of metabolism decreased in the sequence: Atkinson Point > Norman Wells > Pembina > Venezuela. On the other hand, the magnitude of the stimulation of metabolism at high oil concentrations decreased in the sequence: Pembina > Norman Wells > Venezuela > Atkinson Point.

Exposure to light dispersions of Norman Wells crude for longer than 24 hours did not result in a further relative depression of metabolic rate (Figure 23), at least for periods up to 96 hours. During the first 24 hours of experimental exposure both control, and oil-exposed animals exhibited a very pronounced decline in metabolic rate from the normal level, of about 28% and 38%, respectively. This was undoubtedly a metabolic response to short-term starvation. Prior to use the animals were held in large stock tanks and fed ad libitum. It has frequently been observed that in many marine organisms metabolic rate is greatly enhanced by feeding (Newell, 1970). As a matter of routine the animals were not fed during experimental exposures. During the first 24 hours without food the metabolic rate dropped to a non-feeding level. Between 24 and 96 hours the rate declined much more slowly in both control and oil-exposed animals.

During the first 96 hours the general metabolic trends in the two groups were qualitatively similar, although the curve for the oil exposed animals is displaced downward as a consequence of the pollutant stress. After 96 hours the

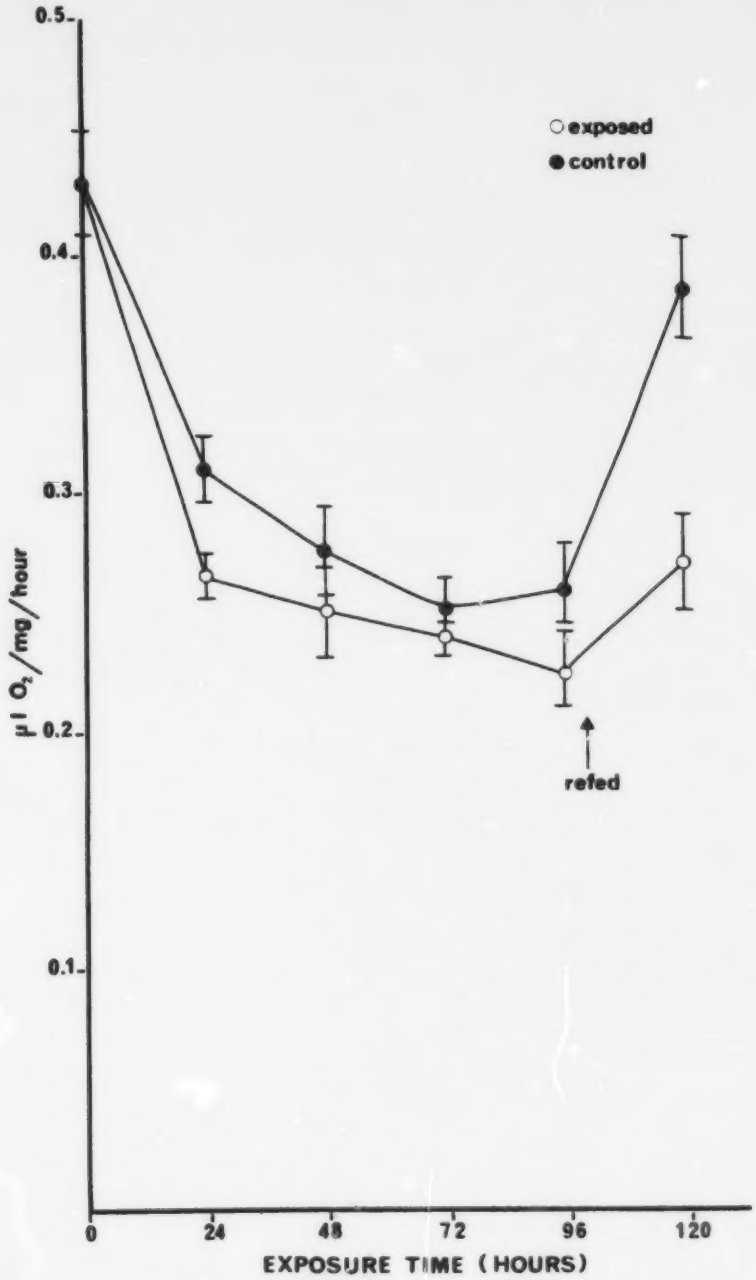

Figure 23. Effect of exposure (at 8°C) for varying time periods to light dispersions of Norman Wells crude oil on the respiratory metabolism (at 8°C) of *Onisimus affinis*. Animals not fed during test, but refed after 96 hours exposure. Oil dispersion replaced every 24 hours. Vertical lines indicate standard errors. (Appendix table 17).

animals were provided with food ad libitum and the metabolic rate was determined 24 hours later. The difference in metabolic rate between control and oil-exposed animals was greatly enhanced following this treatment. The metabolic rate of control animals rose almost to the original pre-test level, while that of the oil-treated animals rose only slightly. This may be a consequence of markedly reduced feeding by the oil-exposed animals. For a discussion of the potential effects of oil pollution on feeding activity refer to section 6.2.3.

The ecological consequence of the nutritional effect noted above is that in a natural field situation, where animals have continuous access to food, the actual difference in metabolic rate between oil-exposed and unexposed animals would be even greater than that indicated by the results of standard laboratory exposure tests, where both groups were starved during the test. It is clear that oil pollution may modify respiration rate both directly by influencing metabolic processes and indirectly by altering the nutritional state of the animal

5.4.1.2 Effect of exposure to oil and dispersant combined

The results of a series of experiments designed to examine the effects on respiratory metabolism of *Onisimus* of pre-exposure to oil dispersions prepared with the dispersant Corexit are presented in Figure 24. Corexit alone, at concentrations comparable to those used with each of the oils, depressed the metabolic rate. At a concentration of 50 ppm (light) the metabolic rate was not significantly different from controls. Increasing the concentration to 500 ppm (medium) resulted in a 30% reduction in metabolic rate that is statistically significant ($p < 0.005$). A further increase in concentration to 2000 ppm did not result in a further reduction in metabolic rate. With Corexit alone there was no evidence of a reversal of inhibition comparable to that observed following exposure to dispersions of crude oils.

However, the reversal of metabolic inhibition with increasing pollutant concentration once again became apparent when animals were exposed to Corexit and crude oils simultaneously. As before, the metabolic rate was consistently depressed following exposure to light or medium concentrations of all three oil/Corexit mixtures, while at higher concentrations the magnitude of the depression declined. A significant

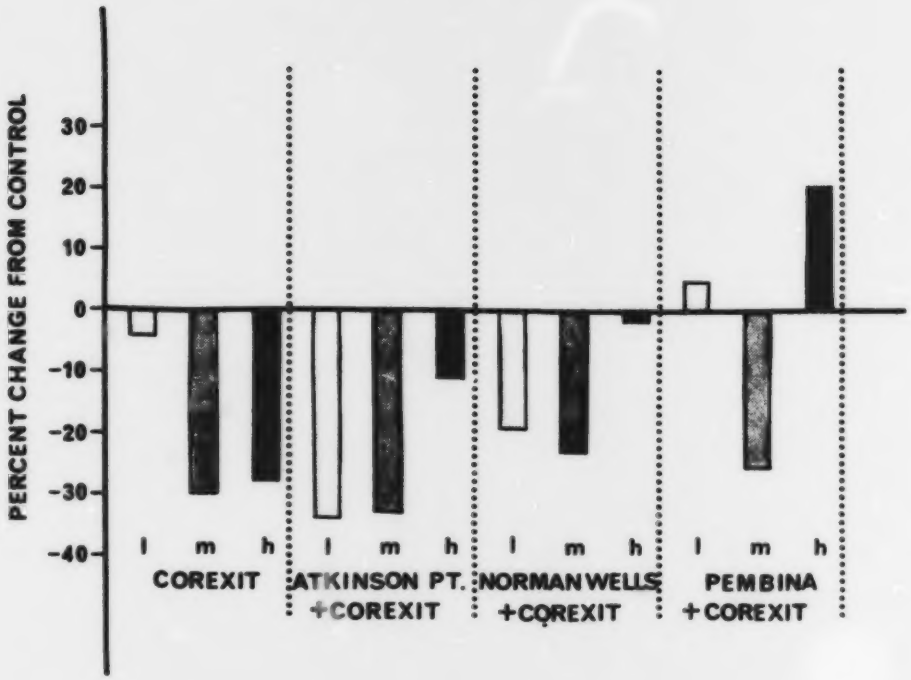

Figure 24. Effect of 24 hours exposure (at 8°C) to various concentrations of Corexit, alone and in combination (1:1) with Atkinson Point, Norman Wells and Pembina crude oils on the respiratory metabolism (at 8°C) of *Onisimus affinis*. (Appendix table 20).

difference in the present instance is that when Corexit was present with the oil the actual magnitude of the reversal was greatly reduced relative to that observed with oils alone. With oil and Corexit mixtures enhancement of metabolism above the control level occurred only with dispersions of Pembina crude and even then not nearly to the degree observed for this oil alone (Figure 24). With both oil alone and oil with Corexit the relative magnitude of the reversals of inhibition were similar for each of the oils tested:

Pembina > Norman Wells > Atkinson Point

In general the maximum metabolic depression observed with the oil/Corexit mixture was slightly greater than that observed with the corresponding oils alone:

	Alone	With Corexit
Norman Wells	-21%	-23%
Atkinson Point	-25%	-34%
Pembina	-20%	-26%
Corexit	-30%	-

5.4.1.3 Effect on metabolism of cell free homogenates

To examine the effect of pre-exposure to dispersions of crude oil on cellular metabolic processes without the complication introduced by indirect effects on activity related metabolism (section 5.4.2) a series of studies were conducted using cell free homogenates of *Onisimus affinis*. The homogenates were prepared from animals that had been pre-exposed to dispersions of Norman Wells crude for 24 hours under standard conditions. In all cases the metabolic rates of the cell free homogenates were enhanced by oil exposure, even at the lowest oil concentrations tested (Figure 25). There was no evidence of a depression of metabolism comparable to that found with intact animals (Figure 22). The enhancement of metabolism ranged from 10% to 45%, and did not vary in a consistent manner with concentration, possibly because the experimental error inherent in working with cell free homogenates is greater than that encountered with intact animals. Comparable studies with the other crude oils have not yet been carried out.

5.4.1.4 Effect of seawater soluble components

Figure 26 is based on data derived from Percy (1974) and is included here because it may shed some light on processes involved in the observed metabolic effects. The data represents changes in metabolic rate of *Onisimus affinis* exposed to various concentrations of seawater soluble components (filtered to remove dispersed particulate material) of Norman Wells and Atkinson Point crude oils. In both cases the general trend is for an increase in

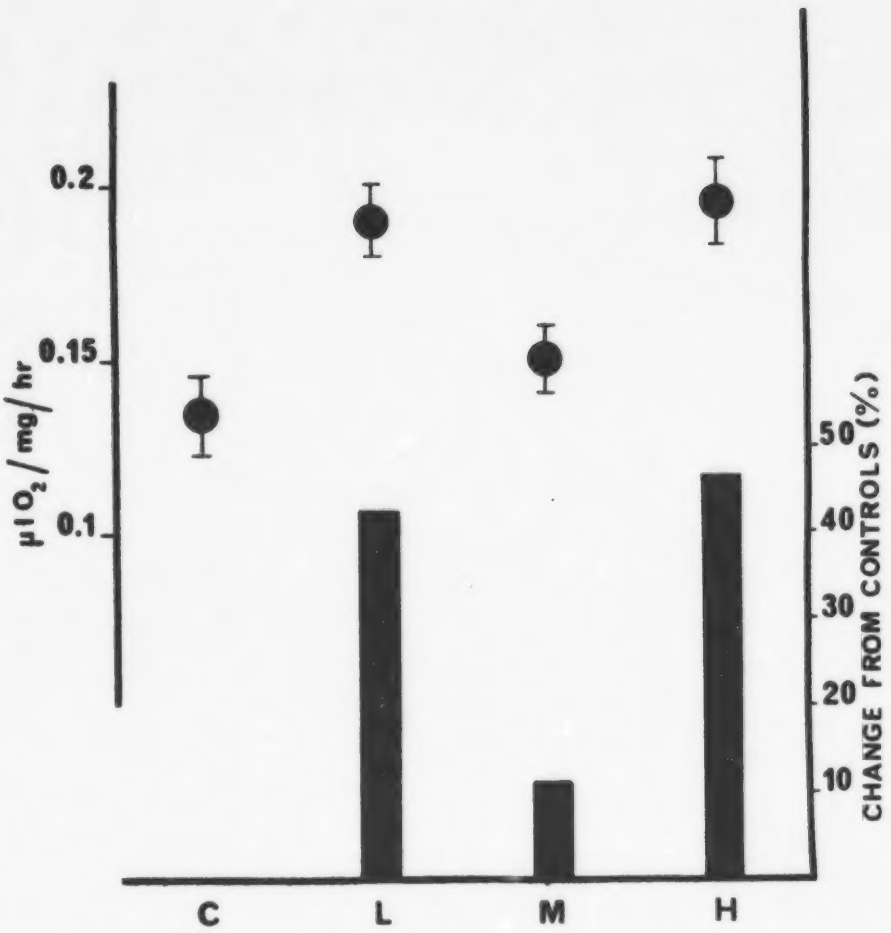

Figure 25. Effect of 24 hours exposure (at 8°C) to light, medium and heavy dispersions of Norman Wells crude oil on the respiratory metabolism (at 14°C) of cell-free homogenates of *Onisimus affinis*. (Appendix table 23).

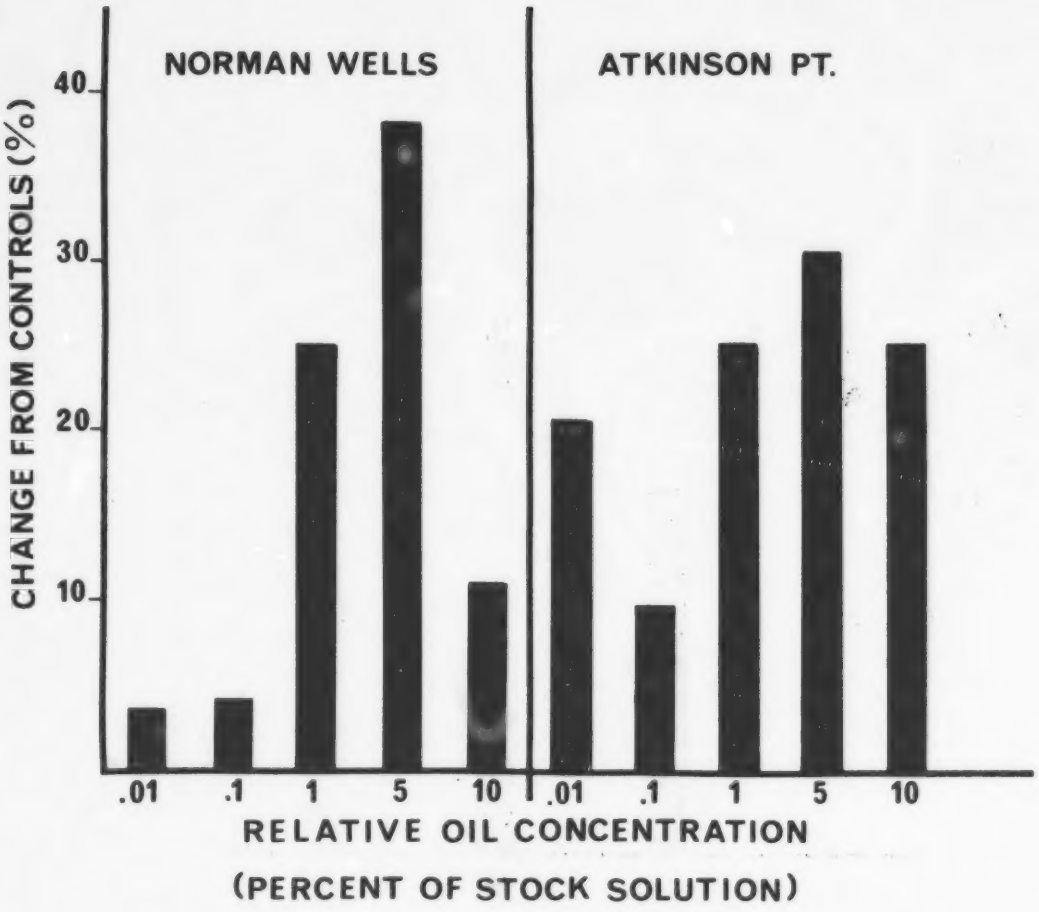

Figure 26. Effect of acute exposure to varying concentrations of seawater extracts of Norman Wells and Atkinson Point crude oils on the respiratory metabolism of *Onisimus affinis* at 7°C. (Appendix 21 and 22).

metabolic rate following exposure to the oil. The magnitude of the enhancement appears to increase as the oil concentration rises, to a maximum of 38% for Norman Wells crude and 30% for Atkinson Point crude. Both maxima occur at the same relative oil concentration. The significance of the decrease in metabolism at higher oil concentrations is uncertain at present. There was no indication of metabolic depression below the level of control animals at any of the concentrations tested.

5.4.1.5 Metabolic effects on other species

Although the most intensive metabolic studies have been focussed on the single species *Onisimus affinis* in an effort to understand the apparently complex effects of oil on respiratory metabolism, preliminary metabolic studies were carried out on several other invertebrate species. Although additional data is required before meaningful generalizations can be made, a number of trends are evident.

The metabolic rate of the amphipod *Atylus carinatus* appeared to be unaltered by exposure to dispersions of Norman Wells crude (Figure 27). In contrast, exposure to dispersed Pembina crude resulted in a depression of metabolism that increased with increasing oil concentration to a maximum of 26%. Within the concentration range tested there was no evidence of a reversal of the metabolic inhibition comparable to that observed in *Onisimus*.

The metabolic rate of the isopod *Mesidotea sibirica* is similarly depressed following exposure to crude oil dispersions (Figure 28). In this instance both Norman Wells and Pembina crudes inhibited metabolism significantly. The maximum depression observed was 35% and 31% for Norman Wells and Pembina, respectively. The fact that the maximum metabolic inhibition occurred following exposure to light or medium dispersions may indicate a reversal of the inhibition, although the trends are not as clear as they were with *Onisimus*.

The metabolic data for the isopod *Mesidotea entomon* is limited at present to that for exposure to dispersions of Norman Wells crude. Over the whole concentration range tested the metabolic rate was enhanced markedly by the oil treatment. However, the increase over the control level was only statistically significant at the highest concentration tested. It is perhaps pertinent to note that the standard deviations of the metabolic rates for oil exposed groups were approximately twice as great for *M. entomon* as for *M. sibirica* (Appendix table 18). Mean metabolic rates and

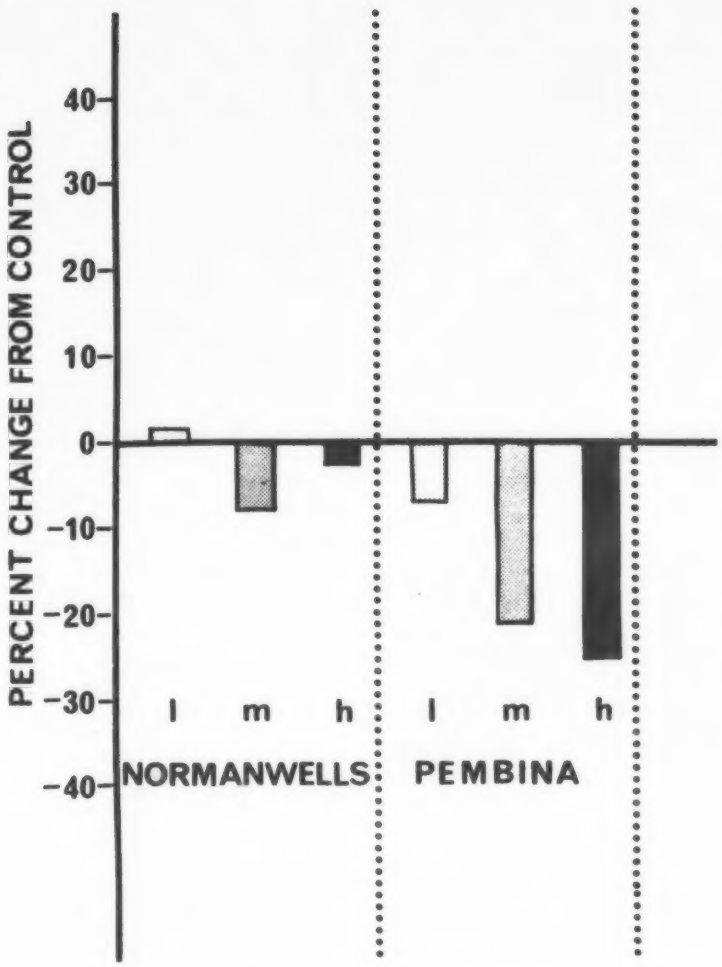

Figure 27. Effect of 24 hour exposure (at 8°C) to light, medium and heavy dispersions of Norman Wells and Pembina crude oils on the respiratory metabolism (at 8°C) of *Atylus carinatus*. (Appendix table 19).

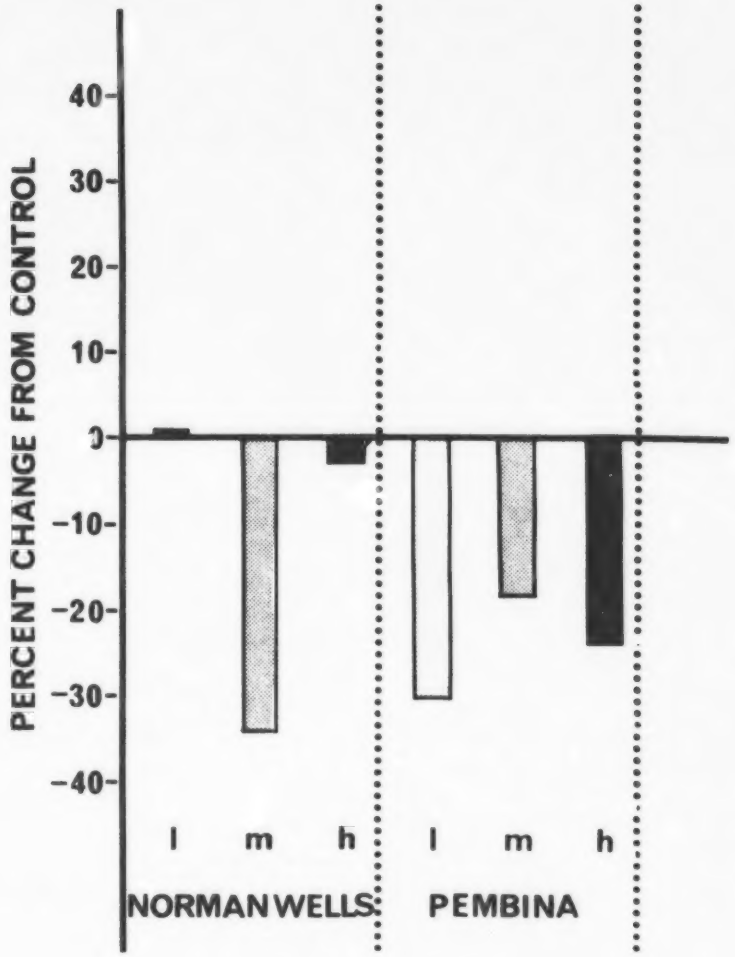

Figure 28. Effect of 24 hours exposure (at 8°C) to light, medium and heavy dispersions of Norman Wells and Pembina crude oils on the respiratory metabolism (at 8 C) of *Mesidotea sibirica*. (Appendix table 18).

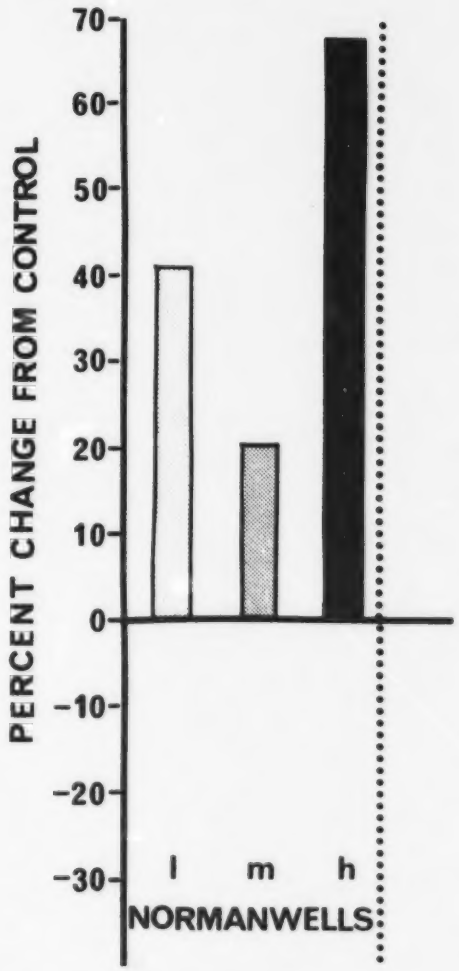

Figure 29. Effect of 24 hours exposure (at 8°C) to light, medium and heavy dispersions of Norman Wells crude oil on the respiratory metabolism (at 8 C) of *Mesidotea entomon*. (Appendix table 18).

standard deviations in control groups were comparable for both species. This suggests that the metabolic response of individual *M. entomon* to oil exposure is more variable than that of individual *M. sibirica*. The precise physiological significance of the differences in response to the oil by these two closely related species is at present uncertain.

5.4.2 Effects on locomotory activity

Results of studies on the effects of 24 hours exposure to dispersions of Venezuela, Norman Wells and Pembina crudes on the locomotory activity of *Onisimus affinis* are presented in Figure 30. Pre-exposure to all three oils resulted in a marked decline in locomotory activity. In each case the magnitude of the depression increased with increasing oil concentration. Exposure to light dispersions reduced activity by 39% to 46%, to medium dispersions by 78% to 96%, and to heavy dispersions by 98%. Exposure to Norman Wells crude appears to have had the most pronounced effect on activity in that locomotory activity virtually ceased following exposure to medium dispersions. The disruption of activity did not appear to be a short-term reversible effect. Animals exposed to Pembina crude for 24 hours and then transferred to clean seawater for a further 24 hours showed no evidence of a recovery of normal activity (Figure 30). It must be emphasized that the activity referred to above is locomotory activity only; even in heavy dispersions the animals exhibited varying degrees of appendage movement, particularly rhythmic beating of the pleopods.

The activity of the medusa *Halitholus cirratus* was monitored by means of an activity score, defined in section 4.8. For tests with Norman Wells, Atkinson Point and Venezuela crude the activity score was routinely measured just prior to exposure to the oil, following 24 hours exposure to the oil and again following 24 hours recovery in clean seawater (Figures 31 and 32). In tests with Pembina crude the activity following recovery was not determined.

Exposure to light, medium and heavy dispersions of Norman Wells crude resulted in a reduction in activity by 45%, 55% and 100% respectively. The disruption of activity in light and medium dispersion was reversible; after 24 hours recovery in clean seawater all of the animals were capable of swimming (Appendix table 27). In contrast, animals exposed to heavy dispersions showed only slight recovery; none of these animals were capable of swimming following return to clean seawater, although 60% of them regained the ability to contract weakly.

The activity of animals exposed to Atkinson Point crude for 24 hours was only minimally effected, even at the highest concentrations tested. A further slight reduction in activity

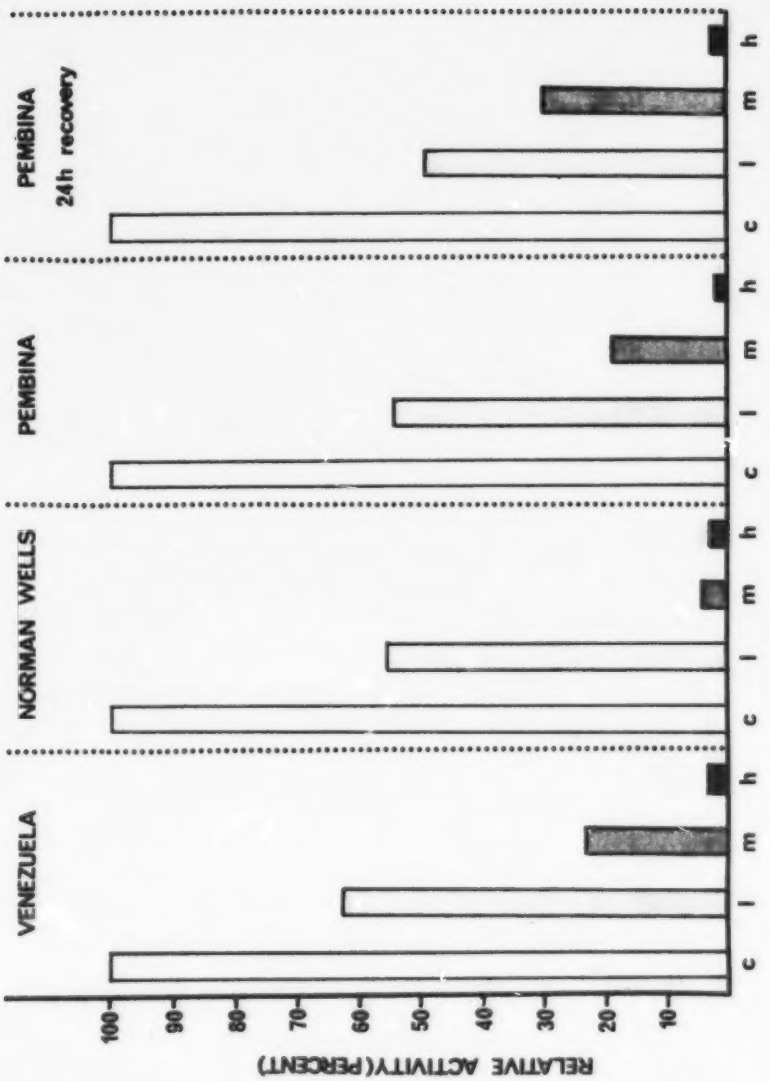

Figure 30. Effect of 24 hours exposure (at 8°C) to light, medium and heavy dispersions of Venezuela, Norman Wells, and Pembina crude oil on the locomotory activity of *Onisimus affinis* Activity of animals exposed to Pembina crude measured again after 24 hours recovery in clean seawater. (Appendix table 25).

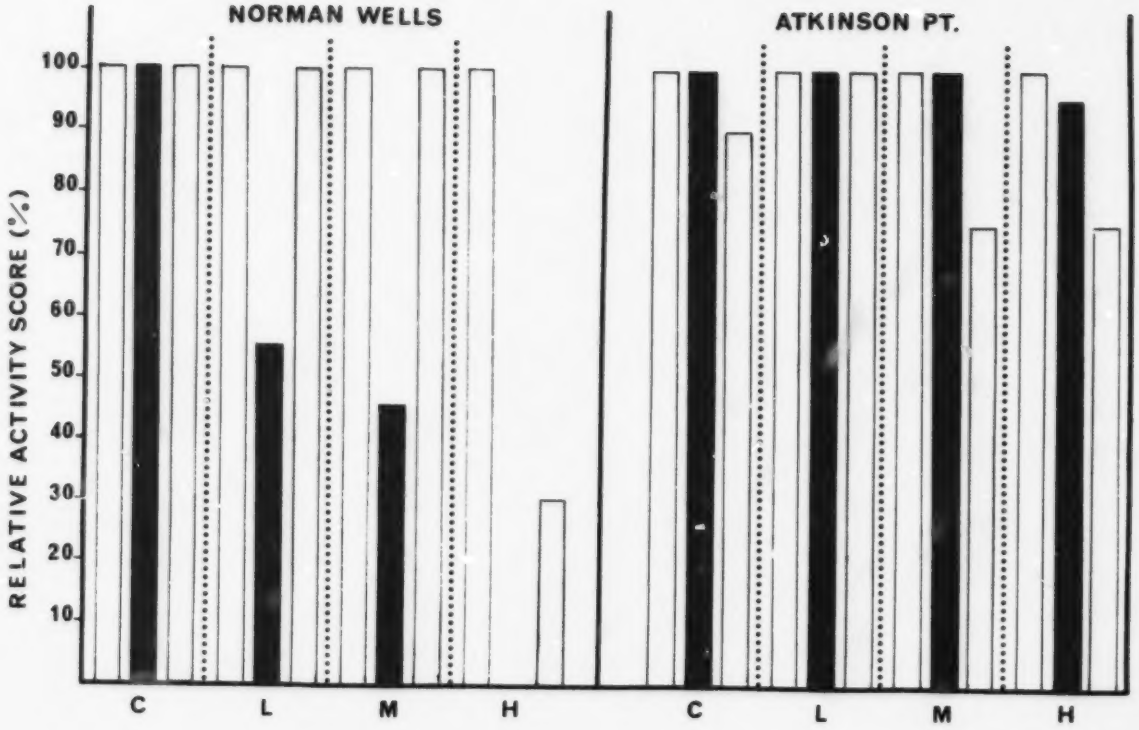

Figure 31. Effect of 24 hours exposure to light, medium and heavy dispersions of Norman Wells, and Atkinson Point crude oils on the activity of *Halitholus cirratus* at 8°C. Activity measured before exposure (white bars), after 24 hours exposure to the oil (black bars), and after 24 hours recovery in clean seawater (grey bars). (Appendix tables 27 and 29).

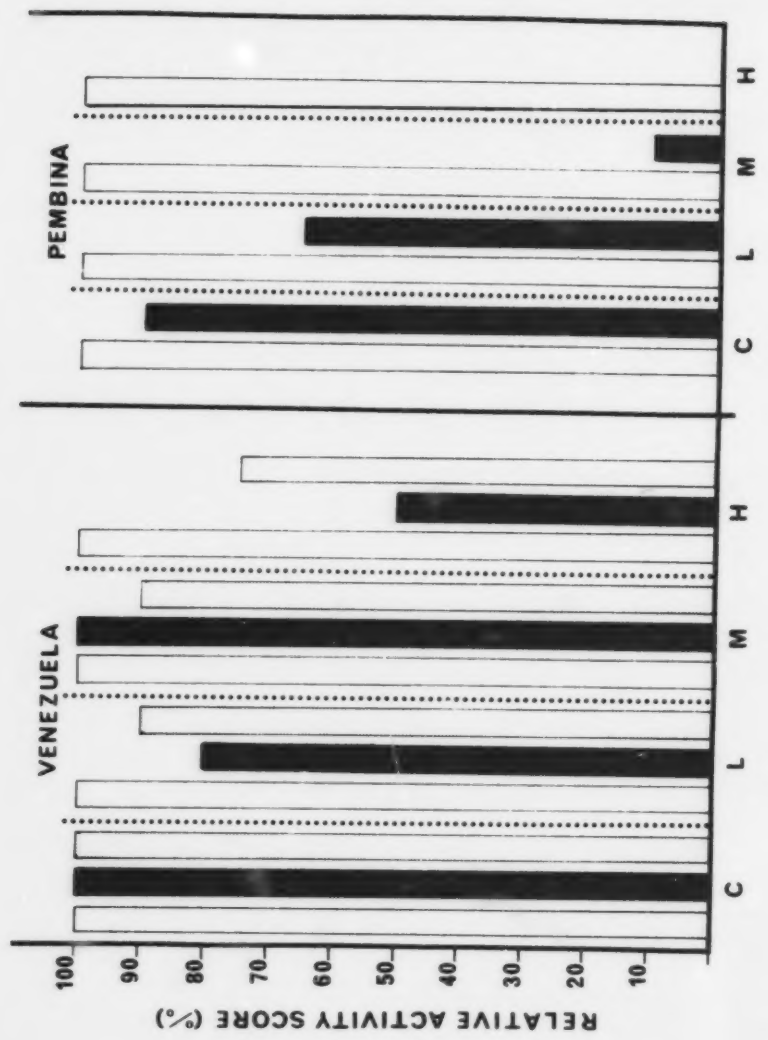

Figure 32. Effect of 24 hours exposure to light, medium and heavy dispersions of Venezuela and Pembina crude oils on the activity of *Halitholus cirratus* at 8°C. Activity measured before exposure (white bars), after 24 hours exposure to the oil (black bars), and after 24 hours recovery in clean seawater (grey bars). (Appendix tables 28 and 30).

score following the recovery period may be indicative of a delayed effect, although the evidence for this is weak at the moment.

Activity was not significantly effected by 24 hours exposure to light and medium dispersions of Venezuela crude The maximum reduction in activity in heavy dispersions was approximately 50%. Activity increased somewhat following the recovery period, but even then only 40% of the animals were capable of swimming normally.

Exposure to Pembina crude had a marked effect on activity; in light, medium and heavy dispersions activity was reduced by 35%, 90% and 100% respectively. In light dispersions only 30% of the animals were capable of swimming normally, while in medium and heavy dispersions swimming was completely inhibited.

The severity of disruption of activity by the different oils decreased in the following sequence:

Pembina > Norman Wells > Venezuela > Atkinson Point

To examine the effect of short term exposure to crude oil groups of *Halitholus* were placed in seawater dispersions (medium) of the four oils and the activity score was determined at hourly intervals for 6 hours (Figure 33). Control animals maintained a uniform high level of activity for the duration of the test. The onset of disruption of activity was rapid in all four oils and maximal inhibition occurred within two to three hours.

The four oils appeared to fall into two distinct groups on the basis of their short-term effects on activity. Atkinson Point and Venezuela crudes reduced activity to about 50% of normal within two hours and continued exposure had little further effect. In contrast Norman Wells and Pembina crudes reduced activity well below 50% of normal (25% and 30% of controls, respectively). Because of the nature of the scoring system, a reduction of activity score of 50% reflects a cessation of swimming activity but not of the ability to contract. Activity scores declining below 50% of normal reflect an increasing inhibition of the contractile response. Thus Atkinson Point and Venezuela crude primarily disrupted swimming ability, possibly by impairing the strength or the coordination of contractions. In contrast, Norman Wells and Pembina crudes not only disrupted swimming, but also inhibited muscular contractions. This is clearly shown in Appendix tables 32 and 35.

A comparison of Figure 33 with Figures 31 and 32 suggests that an adaptation effect may occur with continued exposure to Atkinson Point and Venezuela crudes. Although short-term

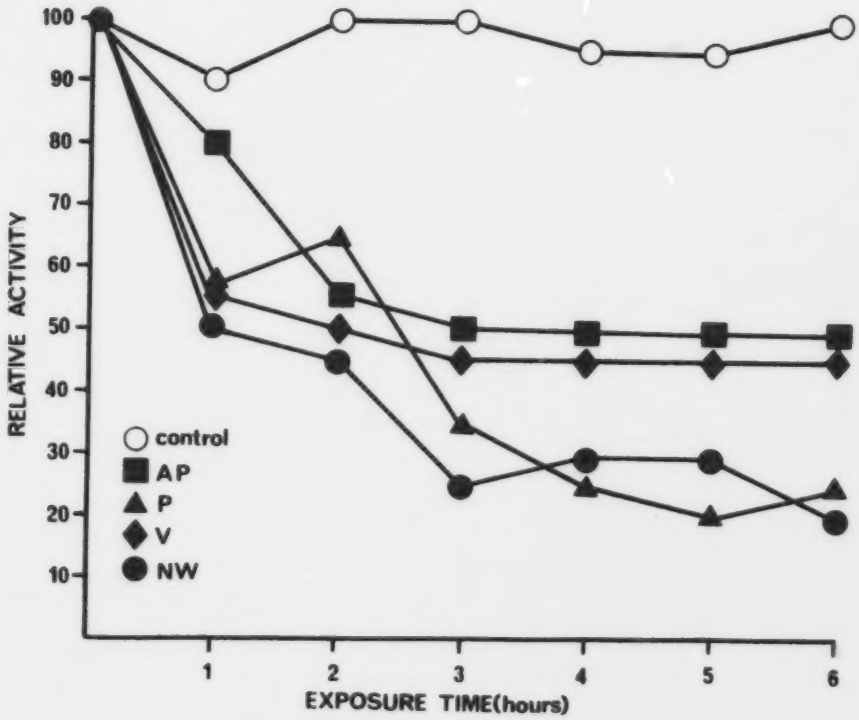

Figure 33. Effect of short term exposure to medium dispersions of Atkinson Point, Pembina, Venezuela and Norman Wells crude oils on the activity of *Halitholus cirratus* at 8°C. (Appendix tables 31, 32, 33 and 34).

exposure reduced the activity score substantially, after 24 hours exposure to the oils the activity returned essentially to normal. A comparable adaptation effect does not appear to occur following exposure to Norman Wells and Pembina crudes. The differences in effect may be related to the disruption of muscular contraction noted above for the latter two oils.

5.5 Sublethal behavioral responses to the presence of oil

5.5.1 Response to oil masses

Results of studies on the attraction to, or repulsion from, nearby crude oil masses by the three crustacean species, *Onisimus affinis*, *Mesidotea entomon* and *Gammarus oceanicus* are presented in Figure 34. As described earlier (section 4.9.1) the affinity coefficient provides a quantitative estimate of the animal's reaction to the presence of the oil. An increasingly positive coefficient indicates an increasing degree of attraction towards the oil, while an increasingly negative value indicates an increasing degree of repulsion from, or avoidance of, the oil mass. In addition to information on the normal response to crude oil, the data for *Onisimus* includes estimates of the affinity coefficients for animals presented with weathered crude oils, and also for animals that had been pre-exposed to dispersions of the appropriate crude oils for 24 hours prior to the affinity tests.

It is immediately clear that the three species differed considerably in their response to the oils. Both *Gammarus* and *Onisimus* exhibited a high degree of avoidance for all three oils. With both species Atkinson Point crude evoked the greatest negative response. Venezuela crude evoked a lesser response with both species, while Norman Wells crude repelled *Onisimus* to a far greater degree than it did *Gammarus*. *Mesidotea* differed significantly from the other two species in that it exhibited an essentially neutral response to Atkinson Point and Venezuela crudes, while Norman Wells crude repelled only slightly (A.C. = -17%). It is perhaps significant that all of the oils evoked either a negative or neutral response; none of the species examined were attracted to any of the oils.

In the case of *Onisimus*, weathering the oil for 24 hours eliminated the avoidance response, both with Venezuela and Atkinson Point crudes. In contrast, the repellent effect of Norman Wells crude was not reduced significantly by comparable weathering. Similarly, pre-exposing the animals to crude oil

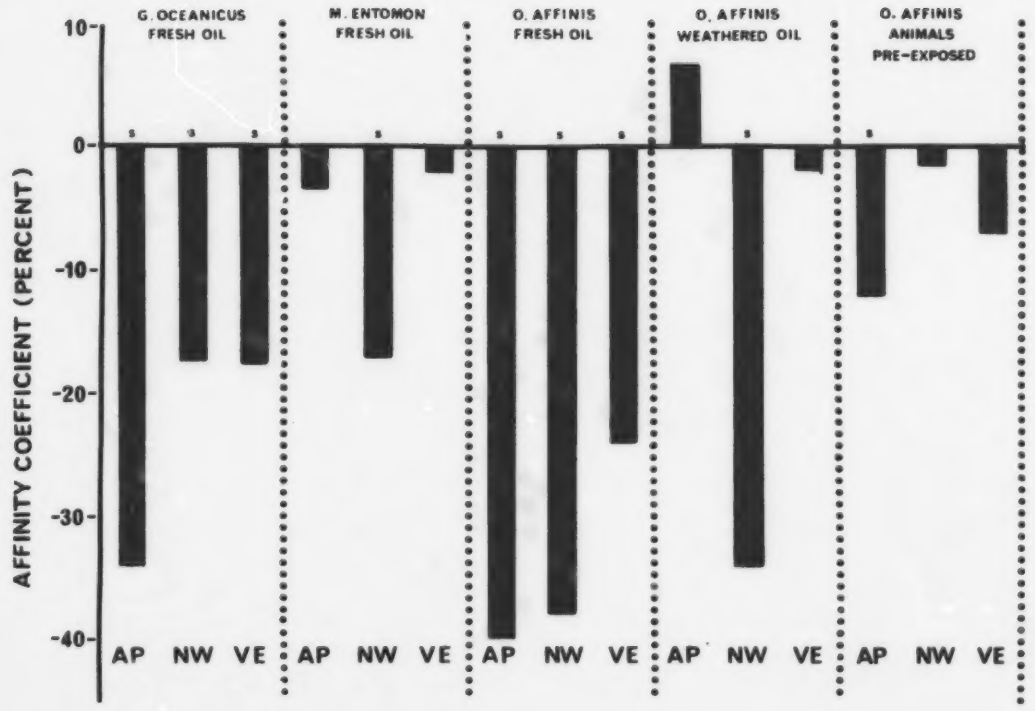

Figure 34. Relative affinity of *Gammarus oceanicus*, *Mesidotea entomon* and *Onisimus affinis* for fresh Atkinson Point, Norman Wells and Venezuela crude oil masses. *Onisimus affinis* also tested with weathered oils and after 24 hours pre-exposure to medium dispersions of the oils. See text for details. (s: significant at the 0.05 level). (Appendix table 35).

dispersions for 24 hours tended to reduce or completely eliminate the avoidance response, suggesting either adaption to the oil or, more likely, damage to chemoreceptors by components of the oil.

5.5.2 Response to oil contaminated food

To examine the effect of oil contamination of food upon the feeding behavior of *Onisimus* and *Mesidotea*, groups of animals were given a choice between untainted and tainted food presented simultaneously. *Onisimus* responded little, if at all, to the tainted food (Figure 35). In contrast, the untainted food was overwhelmingly attractive to all groups tested, with relative feeding scores ranging from 80% to 99%. *Mesidotea*, however, demonstrated an apparent disregard for the presence of oil in the food. The animals responded equally well to both untainted and tainted food as demonstrated by the relative feeding scores of 47% and 53%, respectively. Food contaminated with other oils have not been tested with *Mesidotea* thus far.

The responses of *Onisimus* and *Mesidotea* to oil tainted food when this was the only food available to them are summarized in Figure 36. It is strikingly evident from the data that *Onisimus* was only slightly, if at all, attracted to food tainted with petroleum. Whereas control groups presented with untainted fish responded with a mean feeding score of 80%, those presented with fish tainted with Atkinson Point, Norman Wells and Venezuela crudes, responded with mean feeding scores of only 4%, 5% and 4%, respectively. It should be emphasized that these animals had not been extensively starved prior to use in the tests, but were in a reasonably "natural" nutritional state. It would be interesting to see if the animals reject the oil tainted food so readily if they have been deprived of all food for some time.

Once again, *Mesidotea* responded to the oil in a manner quite different from that of *Onisimus*. Animals presented with untainted fish responded with a mean feeding score of 54%. On the other hand, animals presented with food tainted with Norman Wells crude responded with mean feeding scores ranging from 21% to 39%. Thus, although the response to the oiled food was not as positive as that to "clean" food, nevertheless, substantial numbers of the animals were attracted to the tainted food. The response of *Mesidotea* to food tainted with the other available crude oils has not yet been determined.

5.5.3 Response to oil contaminated sediments

The evident toxicity of oil-tainted sediments to certain species (section 5.3) raises the question of whether or not benthic borrowing species would be attracted to or repelled from areas of sediment contamination, and whether they would, in fact, exhibit normal burrowing behavior in such tainted sediment.

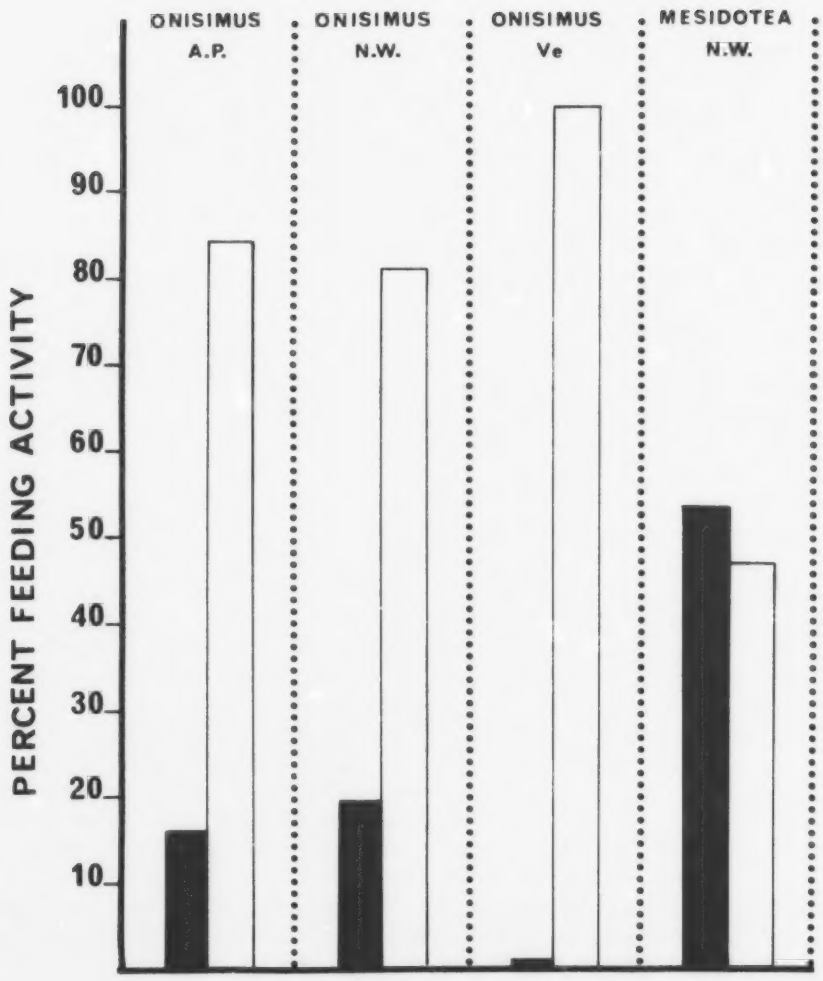

Figure 35. Food choice of *Onisimus affinis* and *Mesidotea entomon* presented with oil-contaminated food (black squares) and clean food (white squares) simultaneously. (Appendix table 42).

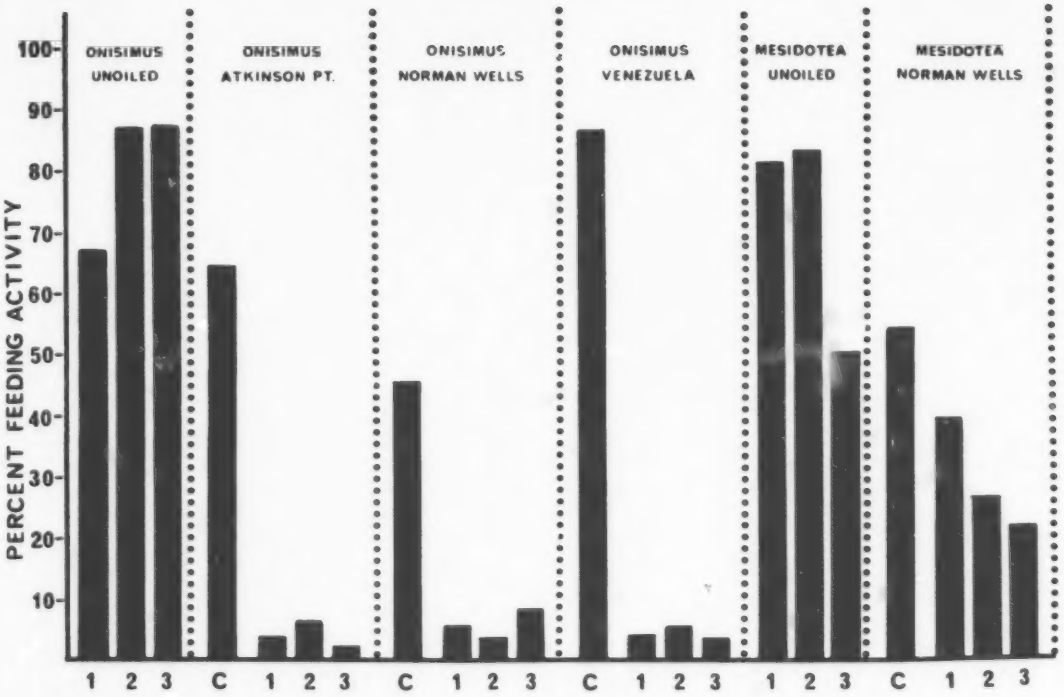

Figure 36. Relative feeding activity of *Onisimus affinis* and *Mesidotea entomon* presented with either clean food (unoiled) or with food contaminated with Atkinson Point, Norman Wells and Venezuela crude oils. Control group (c) and three replicate test groups used for each oil type. (Appendix table 43.)

Our results clearly indicate that different benthic species respond quite differently in the presence of contaminated sediments. Detailed statistical data on preference tests conducted with *Onisimus affinis, Mesidotea entomon, Mesidotea sibirica, and Corophium clarencense* are presented in Appendix tables 36 to 41, inclusive.

Onisimus presented with a choice between clean sediment and sediments contaminated with Norman Wells, Venezuela, Pembina and Atkinson Point crude oils overwhelmingly rejected the oiled sediment (Figure 37). At light, medium and heavy oil concentrations between 80% and 100% of the animals selected the clean sediment. However, in extra heavy oil concentrations the selectivity was markedly reduced or completely abolished. The reponse was most severely reduced in sediments contaminated with Pembina and Norman Wells crudes. It appears that at the highest oil concentration tested the animals lost the ability to distinguish between clean and oil tainted sediments.

It is particularly important to note that maximal avoidance of the contaminated sediments occurred at the lowest oil concentrations tested for all four oils. At the moment we have no information as to the lowest concentrations of oil in sediment to which the animals will respond.

The results in Figure 38 are derived from a duplicate sediment preference test with *Onisimus*. They confirm the marked selectivity of the animals at low oil concentrations, and the reduction in selectivity with increasing quantities of oil in the sediment. As before the reduction is most pronounced with Norman Wells and Pembina crude tainted sediments.

When the oil tainted sediment was permitted to weather in a running seawater system at 5°- 7°C for one week the behavioral response of the animals was significantly modified (Figure 39). The selectivity, particularly with respect to light oil concentrations was markedly reduced. Furthermore the blocking of the avoidance response at the extra heavy oil concentrations was not nearly so pronounced as was the case with the freshly contaminated sediments. Clearly the components of the oil that triggered the avoidance response, as well as those responsible for eliminating the response at high oil concentrations were reduced in concentration in the sediment as a result of the weathering process.

Unlike the unequivocal avoidance response exhibited by *Onisimus*, another benthic amphipod *Corophium clarencense* appeared to be essentially neutral to the presence of oil in the sediment (Figure 40). Only in the presence of Atkinson Point crude was there a consistent avoidance response, but even this was much less pronounced than that observed with *Onisimus*.

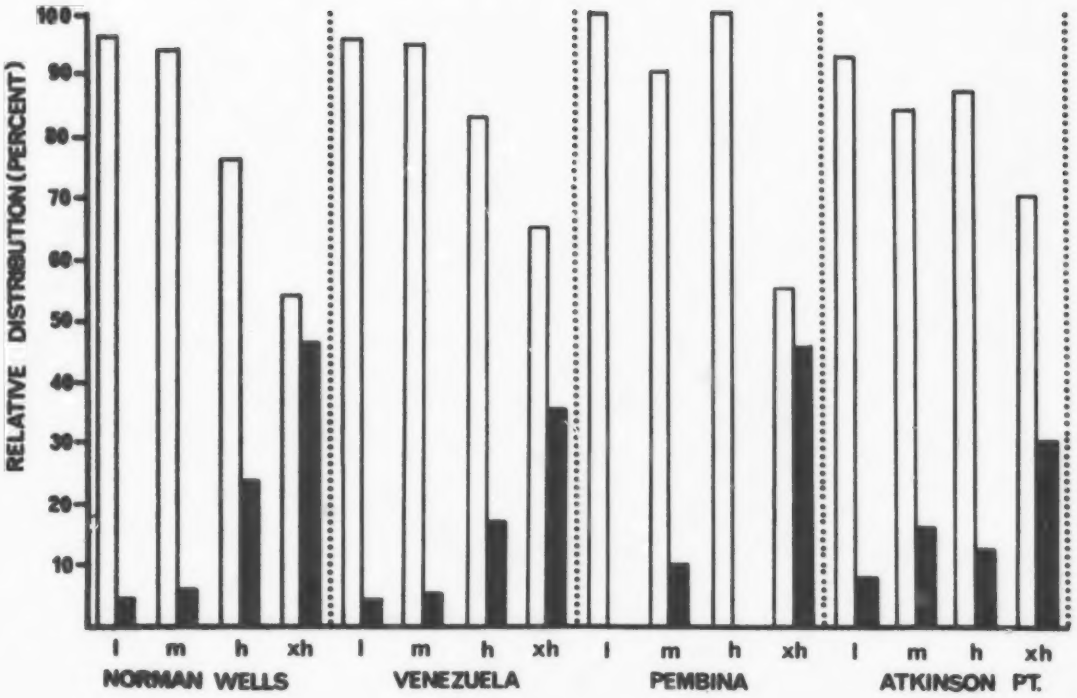

Figure 37. Relative distribution of *Onisimus affinis* presented with a choice between clean sediment (white bars) and sediment contaminated with various concentrations of Norman Wells, Venezuela, Pembina and Atkinson Point crude oils (black bars). Degree of contamination of sediment indicated as light (l), medium (m), heavy (h) and extra heavy (xh), corresponding to 0.05, 0.5, 1.0 and 2.0 ml of oil/15 grams dry sediment, respectively. Series A. (Appendix table 36.)

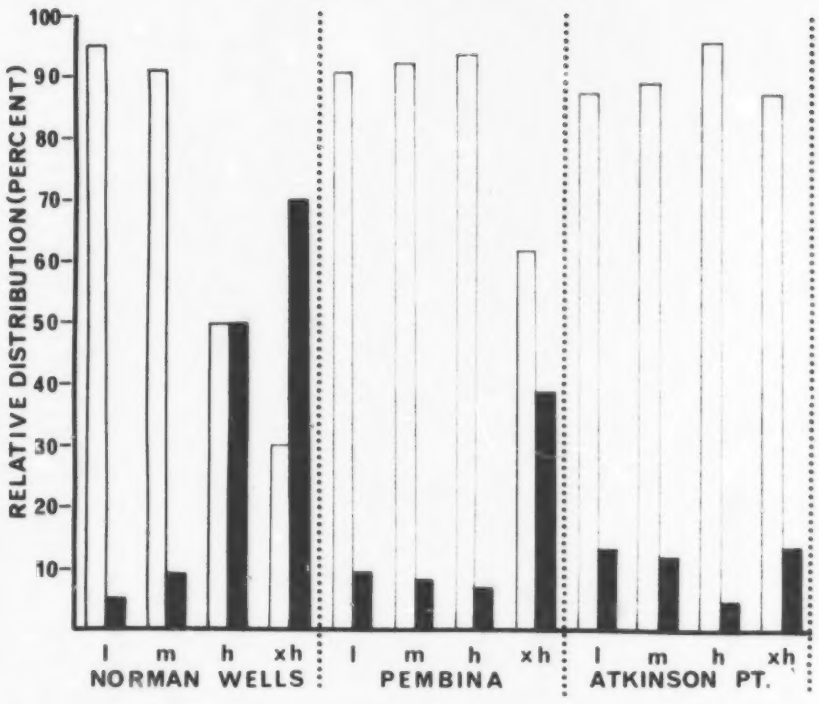

Figure 38. Relative distribution of *Onisimus affinis* presented with choice between clean sediment (white bars) and sediment contaminated with various concentrations of Norman Wells, Pembina and Atkinson Point crude oils (black bars). Degree of contamination of sediment indicated as in Figure 37. Series B. (Appendix table 37).

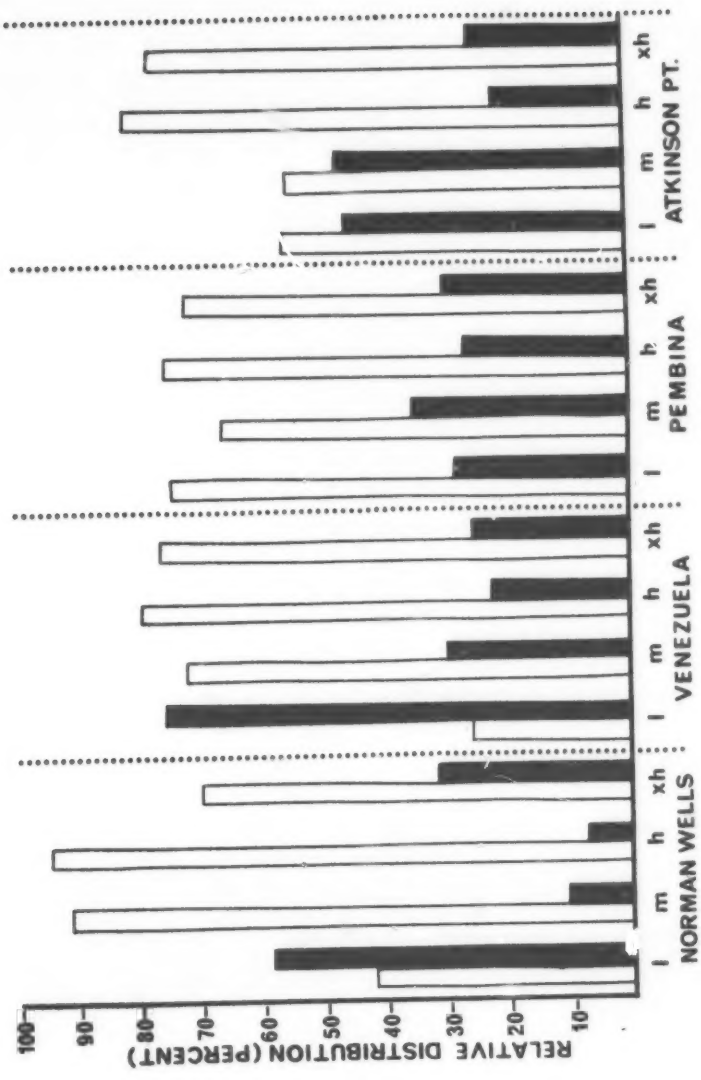

Figure 39. Relative distribution of *Onisimus affinis* presented with choice between clean sediment (white bars) and sediment contaminated wich various concentrations of Norman Wells, Venezuela, Pembina and Atkinson Point crude oils (black bars) and permitted to weather at 5° - 8°C in running seawater system for one week prior to test. Degree of contamination of sediment indicated as in Figure 37. (Appendix table 38).

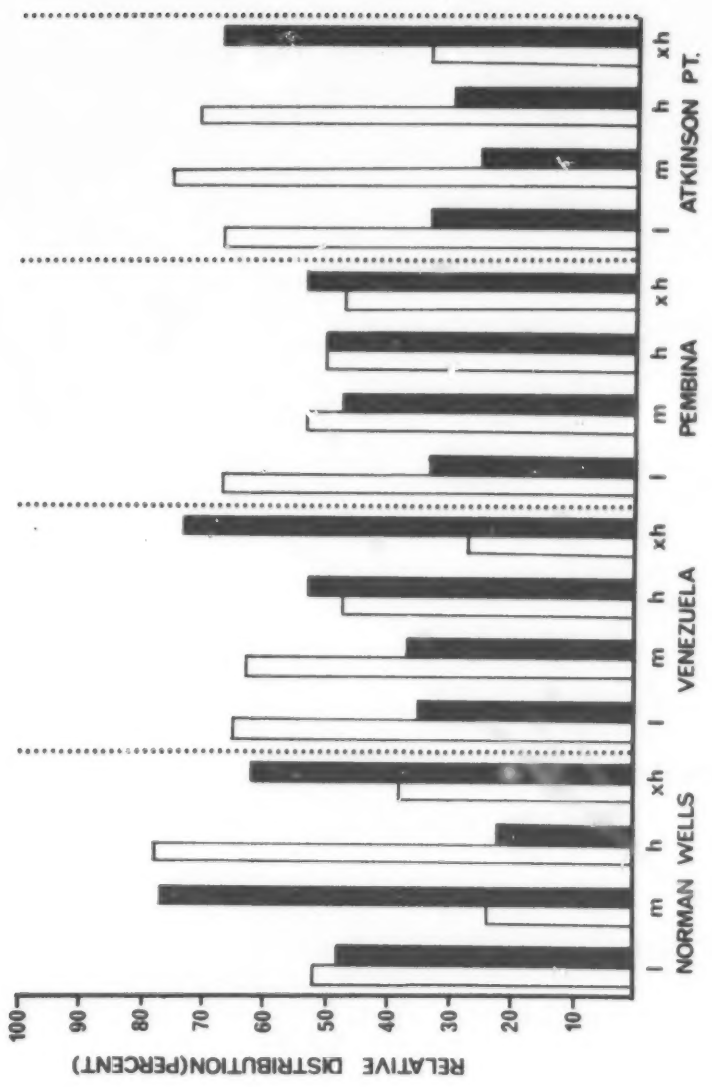

Figure 40. Relative distribution of *Corophium clarencense* presented with choice between clean sediment (white bars) and sediment contaminated with various concentrations of Norman Wells, Venezuela, Pembina and Atkinson Point crude oils (black bars). Degree of contamination of sediment indicated as in Figure 37. (Appendix table 41).

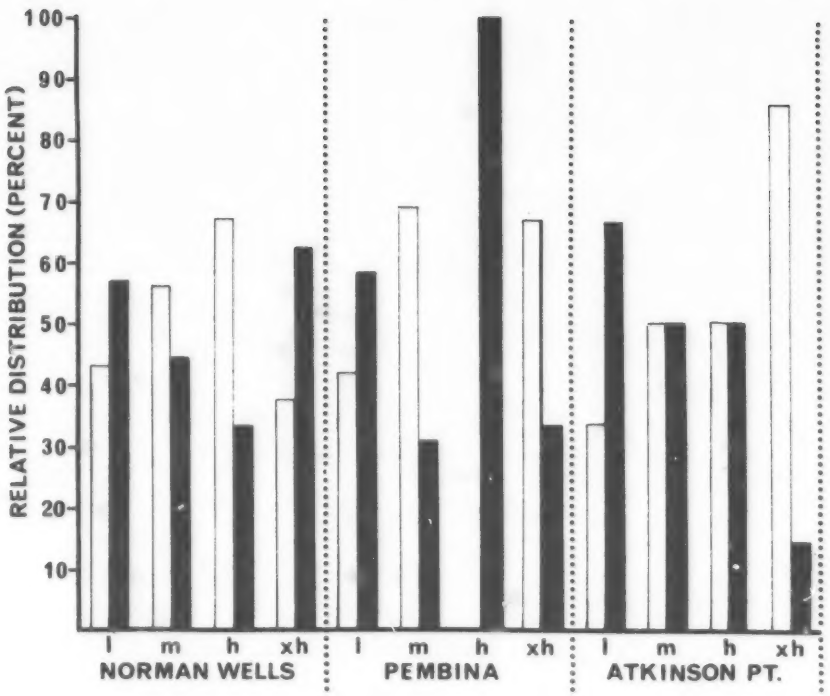

Figure 41. Relative distribution of *Mesidotea entomon* presented with choice between clean sediment (white bars) and sediment contaminated with various concentrations of Norman Wells, Pembina and Atkinson Point crude oils (black bars). Degree of contamination of sediment indicated as in Figure 37. (Appendix table 39).

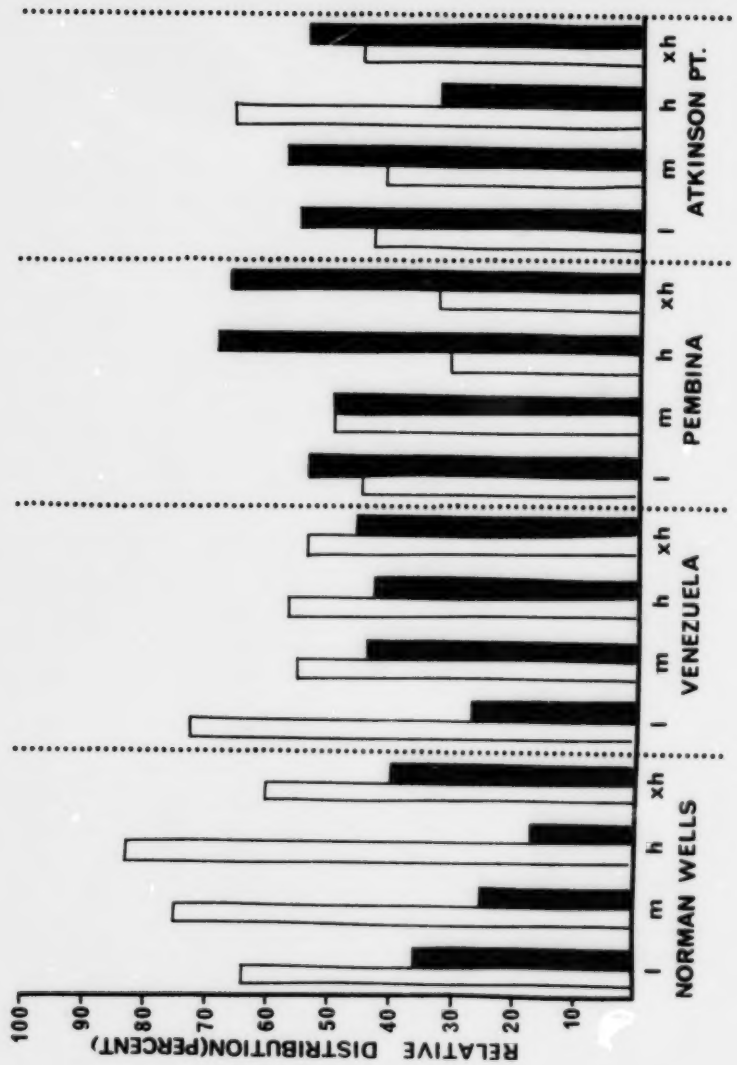

Figure 42. Relative distribution of *Mesidotea sibirica* presented with choice between clean sediment (white bars) and sediment contaminated with various concentrations of Norman Wells, Venezuela, Pembina and Atkinson Point crude oils (black bars). Degree of contamination of sediment indicated as in Figure 37. (Appendix table 40.)

Both *Mesidotea entomon* and *Mesidotea sibirica* were similar to *Corophium* in that little consistent preference for oil-tainted or untainted sediments could be demonstrated (Figures 41 and 42). A possible exception occurred in the case of exposure of *M. sibirica* to sediments tainted with Norman Wells crude, where there was some evidence of a significant avoidance response.

6. DISCUSSION

6.1 Habitat type and the manner of oil exposure

6.1.1 The introduction of oil into different habitats

As was pointed out in section 3.2, the ecological impact of an oil spill is strongly influenced by a wide range of interacting variables, both physical and biological. Although these diverse variables have an important bearing on the magnitude of the impact, from the strictly biological point of view the immediate consequences are a function of the direct encounter of individual organisms with the oil in their particular habitat. However, because of its physical and chemical characteristics crude oil does not spread uniformly through the various types of habitats constituting the marine environment. Not only the quantity of oil, but also the particular physical form in which it occurs will vary quite considerably and unpredictably in different habitats. To realistically assess the potential effects of oil on animal populations it is therefore, essential to examine individually the several distinct types of habitats into which the oil is likely to spread and to consider the dominant physical form and probable dose of oil that could occur in each of these specific habitats. Only with this approach is it possible to relate the experimentally demonstrable lethal and sublethal physiological effects of exposure to oil to situationsthat could occur in the natural environment.

A detailed discussion of the hydrodynamics of a submarine well blowout is presented in Beaufort Sea Technical Report #33. Gas and oil emerge from a well head at very high velocities and will entrain large quantities of seawater into a turbulent bubbler type plume. Some of the oil will be dispersed as fine droplets and will remain suspended in the water column to be transported away from the spill site by water currents. Most of the oil will inevitably coalesce at the surface and in open water will rapidly spread to form a drifting slick. The ultimate fate of the slick and of the oil entrained in particle form in the water column will be

governed largely by hydrographic, physiographic and climatic factors (section 3.2). The spread of spilled oil, in a variety of physical forms, into several ecologically important areas of the Beaufort Sea coastal marine ecosystem is outlined schematically in Figure 43. Each of these principal habitat types will now be considered in turn.

6.1.2 Oil in the intertidal habitat

The most dramatic and obvious biological damage associated with oil spills in temperate and tropical waters occurs when drifting slicks are driven ashore and blanket large areas of the biologically rich intertidal zone. In most areas of the western Arctic the intertidal zone is depressingly barren of marine life as a result of intense scouring by sea ice. Damage to invertebrate fauna in these areas will consequently be minimal. Such stranded oil is, however, still available for re-introduction into other areas of the marine environment, and should not be considered as completely inocuous.

6.1.3 Oil in the sub-ice habitat

Oil released under ice, or transported there by currents will tend to accumulate in irregularities in the ice undersurface to form oil lenses of variable size (Keevil and Ramseier, 1975). Accumulation of significant quantities of oil in this under-ice habitat may have a number of very important ecological consequences.

A rich algal bloom forms on and within the lower surface of annual ice in spring (Apollonio, 1961; Horner and Alexander, 1972). This ice flora "forms an important fraction of the total production in the Arctic Ocean and....it helps greatly to prolong the productive season beyond that of the water-borne phytoplankton" (Dunbar, 1975). In addition, a variety of marine invertebrates have been observed congregating in the general vicinity of the ice-water interface (Green and Steele, 1975; Percy, 1975). The algal bloom may serve as an important food source for some species during the spring, a time when phytoplankton levels in the water column are very low.

Although the algal components of such sub-ice communities have received considerable attention recently little is known about the potential impact of crude oil on these phytoplankton. Virtually nothing is known regarding the general ecology and trophic inter-relationships of the animal components of this community and little is known about the sensitivity of the animals to crude oil. The impact of oil on organisms in the sub-ice habitat may, in some respects, be similar to the impact on intertidal fauna in other areas. In both situations

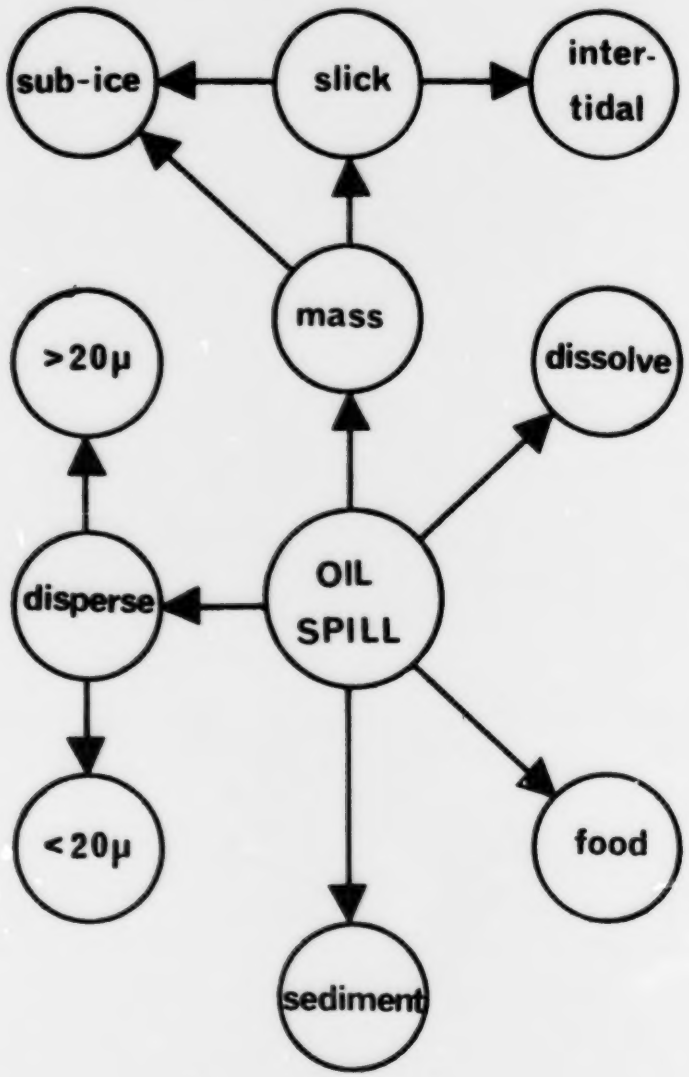

Figure 43. Spread of spilled crude oil into various parts of the arctic marine ecosystem. See text for details.

animals are likely to be subjected to smothering and fouling by viscous oil masses. Oil-fouled animals are unlikely to recover, even if they do succeed in escaping from the oil (Percy, 1974). The attraction-repulsion response to oil masses trapped in pools on the undersurface of the ice could be a decisive factor in the survival of certain species. Both amphipod species examined thus far were clearly repelled by all the oils tested, although the degree of avoidance varied somewhat with the oil type. As the oil is weathered it appears to become less distasteful; however, weathering appears to be minimal in sub-ice oil. It is significant that none of the species examined thus far were attracted to oil masses. The behavioral responses will be considered in more detail in section 6.2.3.

A particularly disturbing aspect of the accumulation of oil in pockets under and within the sea ice is that the trapped oil does not appear to weather significantly over the course of many months. The reason generally cited for the relatively limited impacts of crude oil spills in temperate and tropical waters is that the oil weathers very rapidly and loses most of the toxic components while drifting as a slick upon the sea surface. This appeared to be the case in the Torrey Canyon oil spill (Smith, 1970). Clearly this does not happen with Arctic under-ice oil spills. In the present study, the toxicity of oil recovered from the ice 6 months after being spilled was not significantly lower than that of fresh oil. Studies reported in Beaufort Sea Project Technical Report #27 also indicate that the recovered oil is virtually indistinguishable from fresh crude with regard to viscosity, density, gas chromatographic profile and other parameters.

This severe reduction in the rate of weathering of trapped oil has two potentially very important ecological consequences; it provides a means of transporting the oil, in both a spatial and a temporal sense, without significant diminution of toxicity. Natural ice movements in the Beaufort Sea Gyre could result in the oil being transported considerable distances from the spill site and reintroduced into the water column, still in a toxic form. In other words, there would be little decrease in toxicity with increasing distance from the spill site, as occurs in ice-free waters.

Perhaps of even greater significance is the fact that the oil from a prolonged late summer blow out would accumulate within and under the ice over a considerable period of time. There would be little or no diminution of toxicity with

increasing time after release into the environment, as occurs in ice-free waters. Toxic components of the accumulating oil would be preserved unchanged until the spring break up, when much of the oil could be re-introduced into the water column en masse. In view of the initiation of intense biological activity in the water column during the post-break-up period this may be the worst possible time of the year for the reintroduction of unweathered oil into the seawater. Planktonic larval stages of many species may be particularly vulnerable at this time of year. Larvae of many species of crustaceans are killed very rapidly when exposed to oil concentrations of 100 ppm, and significant mortality and sublethal effects are evident at very much lower concentrations (Mironov, 1969; Wells, 1972). In contrast, many bivalve larvae have been found to be particularly tolerant of crude oil, with detectable adverse effects only occurring at concentrations in excess of 1 ppt (Renzoni, 1973).

6.1.4 Oil in the neritic habitat

Oil may occur in the water column in both a dispersed and a dissolved state, and it is in these forms that it is likely to spread most widely through the ecosystem and interact with the greatest number of species. It is difficult to differentiate between biological effects resulting from dissolved and finely dispersed components of the oil. Initially much of the oil occurs in the dispersed form, but increasing quantities of particular components of the oil dissolve in the seawater (Anderson et al., 1974). It is generally agreed that the finely dispersed oil is more toxic to marine life than are soluble extracts. This may, however, be largely due to the fact that much higher concentrations of oil occur in seawater in a dispersed form than in a dissolved form (section 5.1.2.5). If the oil droplets are less than 20μ the mixture is considered an emulsion, and if greater than 20μ, a suspension. The degree to which dispersion of the oil occurs depends upon the turbulence of the seawater. The droplets, once formed can be rapidly dispersed over a considerable distance and to great depths as reported by Freegarde (1968) and Conover (1971). Several days after the wreck of the tanker 'Arrow', subsurface particulate oil was detected some 250 km from the scene of the spill (Forrester, 1970). Berridge et al. (1968) point out that when water is the continuous phase there is no real limit to the extent to which the droplets are able to disperse.

Little useful information is available about the concentrations of oil to which arctic neritic species might be exposed, and for what length of time; both critical factors in assessing

potential toxic effects. Most studies to date have involved oil suspended in seawater by dispersion from a surface slick. Water samples collected 0.25 m beneath a slick two days after a 1000 barrel crude oil spill contained 800 µg/l, most being in a particulate form (Gordon et al., 1973). In this same paper, a review of available information on oil concentrations in seawater under laboratory conditions reveals values ranging from 267 to 1453 µg/liter. Anderson et al. (1974) reported oil concentrations of 4.7 ppm persisting for up to 72 hours in seawater media. In the present study mean oil concentrations after 24 hours in light and medium dispersions ranged from about 5 to 20 ppm. Heavy dispersions formed under conditions of unnaturally violent agitation (blender) had oil concentrations ranging from 30-200 ppm after settling for 24 hours.

Presumably even greater quantities of dispersed oil than that reported above beneath a surface slick would initially be entrained in the water column following a submarine blow out. In the case of a 65,000 barrel spill from a drilling rig in the Mississippi delta, the concentration of dispersed oil in the water column ranged from 1-70 ppm within a one mile radius of the spill (McAuliffe et al., 1975). The concentration of dissolved hydrocarbon in the same area ranged from 0.001 to 0.2 ppm.

On the basis of the short-term lethal studies most of the species appeared to be relatively tolerant of high concentrations of dispersed crude oil. Even with the so called "sensitive" species dispersed oil concentrations of 100 ppm or greater were required to cause significant mortality during four days continuous exposure. It is unlikely that such high concentrations will occur widely in the water column, but may be reached in limited areas in close proximity to a spill, or where large accumulations of sub-ice oil are released into the seawater at break up.

It should also be kept in mind in extrapolating laboratory results to the field situation that the experimental toxicity tests reported here were routinely carried out with fresh crude oil that was replaced at 24 hour intervals. Consequently, the experimental results are based on the worst possible exposure conditions that could conceivably occur. Exposure of the oil at the sea-air interface for any length of time would undoubtedly result in a gradual reduction in toxicity through weathering.

In view of the above considerations it appears certain that massive short-term mortality among adult organisms inhabiting the neritic zone, as a consequence of contact with particulate or sub-particulate crude oil will, if it occurs at all, be limited to relatively small areas. Furthermore, only

particularly sensitive species are likely to be severely affected over the short-term, within these areas.

The formation of toxic dispersions of oil in the water column is greatly facilitated by the application of chemical emulsifiers. The toxicity of the resulting oil/dispersant mixture may be greater than (Perkins, 1968), the same as (Van Wiele, 1968), or less than (Nelson-Smith, 1970) that of the oil by itself. In many cases much of the toxicity is associated with the dispersant. However, even the use of modern low toxicity dispersants cannot eliminate the inherent toxicity of the crude oil. It is clear that "there are no dispersants that are not toxic in the presence of oil" (Blumer, 1970).

In the present study emulsification of the oil with the dispersant Corexit appears to increase the toxicity of crude oils to *Onisimus* only slightly, if at all. The emulsifier by itself did not cause significant mortality at the concentrations tested. That the compound is not as inocuous as the lethal bioassays indicate, however, is suggested by the marked interference with respiratory metabolism; a point that will be considered further in section 6.2.2.

It is probable that the increase in toxicity of oil/emulsifier mixtures reported in some cases is attributable simply to entrainment of greater quantities of oil into the water and stabilization of the resulting dispersion. Since in the present study we employed particularly violent agitation to produce finely dispersed semi-stable dispersions of oil alone, it is probable that addition of the emulsifier served only to stabilize the dispersion somewhat, but did little to increase the quantity of oil entrained in the dispersion. This could account for the observed lack of effect of Corexit on oil toxicity.

In addition to increasing the quantities of particulate oil in the water column, the use of emulsifiers also results in the formation of a larger proportion of smaller-sized droplets (Stokes and Harvey, 1973). Because of their action in increasing the particulate oil concentration in the neritic habitat emulsifiers should be used to disperse oil spills only as a last resort. Tarzwell (1973) characterizes the use of dispersants in oil clean up operations as "something like sweeping dirt under a rug."

6.1.5 Oil in the benthic habitat

The high sediment load carried by the Mackenzie River into the Beaufort Sea, in the form of a massive and visually impressive plume, poses special problems in terms of the consequences of a

major oil spill in the area. The Santa Barbara blowout in California proved rather instructive in this respect. Heavy rains prior to this spill resulted in a heavy run-off that produced large sediment plumes in inshore areas. Oil slicks penetrated only short distances into such plumes and much of the oil was adsorbed on suspended particles and carried to the bottom (Kolpack, 1971). In effect, the sediment acted as a natural sinking agent. At first glance this might appear to be a rather effective self-purification process. In fact, it appears to compound the problem immeasurably. Unlike an oil slick, oil bound to bottom sediments is essentially beyond human control. Sinking does not remove the oil from the ecosystem, rather it concentrates it in one ecologically important part of the marine environment.

Little is known about the effect of such oil-contaminated sediments upon benthic epifauna and infauna. The present studies indicate that certain benthic species are killed by short-term exposure to oiled sediments, but only at very high oil concentrations; concentrations unlikely to be realized in the natural environment, except perhaps in close proximity to a spill. At present we have no information on possible toxic effects resulting from prolonged exposure to sediments tainted with lesser quantities of oil.

Of considerable ecological concern is the fact that certain species are capable of detecting low concentrations of oil in the sediment. Behavioral patterns may be markedly altered in the presence of such tainted sediment. The ecological consequence of the contaminated sediment avoidance response exhibited by *Onisimus* is at present uncertain. Animals may migrate from areas of contaminated bottom to adjacent clean areas. However, a tendency to avoid burrowing in oil-tainted sediment may result in certain benthic species being subjected to increased predation. Not all species avoid contaminated sediments. It is perhaps significant that although several species showed no particular preference for clean or tainted sediments, there was no evidence of a consistent attraction to tainted sediment by any of the species tested.

Once oil is bound into superficial bottom sediments it is likely to have a long term effect on the benthic community. Studies in temperate areas suggest that oil trapped in sediments is degraded very slowly (Blumer and Sass, 1972). Given the sub-zero temperatures of arctic marine sediments the rate of degradation may be even slower. The present study indicates furthermore that the rate of decline in toxicity of sediment-bound oil varies markedly with different oil types.

Oil contaminated bottom sediments may not be static. Sediments in the Beaufort Sea are intermittently eroded and redeposited by bottom currents (Pelletier, 1974), so that

the potential exists for contamination of a much wider area of sea floor than that affected by the initial deposition. In the Santa Barbara spill, oil initially deposited in shallow water was later transported and redeposited in deeper areas (Kolpack, 1971).

6.2 The biological effects of oil exposure

6.2.1 Short-term lethal effects

The usefulness of short-term lethal toxicity tests as indicators of potential environmental damage is limited by the fact that they measure only a rather gross physiological effect, namely the death of the organism. They can nevertheless provide some information about the relative sensitivities of various species to pollutants such as crude oil. Such information is a prerequisite for assessing potential short-term effects of pollution incidents as well as for charting the general direction of more intensive research on oil effects. Particularly sensitive species can sometimes prove useful as valuable biological indicators of environmental quality. Precautions required in the conduct of such toxicity tests have been discussed at length by Perkins (1972).

On the basis of our short-term lethal studies most of the species examined appeared to be relatively tolerant of high concentrations of crude oils. Different oil types varied considerably in toxicity. In general, Venezuela and Norman Wells crudes proved to be more toxic than either Atkinson Point or Pembina crude, although species differences were evident. This supports the observation of Ottway (1970) that "the blackest and thickest crude oils are the least toxic, while the translucent thin, brown oils are the most toxic." The very complex and variable composition of crude oil accounts for the considerable differences in toxicity observed for different oil types. The various components of crude oil differ markedly in their degree of toxicity. Much of the toxicity appears to be associated with certain of the aromatic fractions boiling below 149°C (Ottway, 1971). The manner in which marine organisms may be killed by crude oil has already been discussed in section 3.3.2.

As expected, the various species differed considerably in their ability to survive in oil dispersions. Relative species sensitivity to oil pollution has already been discussed at length in section 3.2.10. Isopods of the *Mesidotea* complex proved particularly resistant to all oils tested. These animals are abundant in the inshore Beaufort Sea and appear to form a

significant component of the diet of certain fish species. Surprisingly, the planktonic copepod *Calanus hyperboreas* tolerated exposure to oil dispersions rather well over the short-term, even at the highest concentrations tested. Even animals that became trapped in the surface slick and totally coated with oil generally survived for at least 96 hours. Such planktonic forms have often been found to be particularly sensitive to oil products. Mironov (1969) working with zooplankton from the Black Sea reported that death of the animals was accelerated at oil concentrations as low as 0.001 ml/L., while at concentrations of 0.1 ml/L all animals died within one day. Clearly more work will be required on zooplankton species before it will be possible to make meaningful generalizations concerning oil impact.

6.2.2 Sublethal physiological effects

The short-term lethality studies tend to indicate a rather high tolerance level for crude oil among the various species examined. However, a closer examination of the more subtle sublethal effects upon physiological functioning suggests that the oil may not be quite as benign as it first appears. Such sublethal effects may directly result in the death of organisms over an extended period, or more insidiously may impair the animals ability to withstand normal environmental stresses. Two measures of physiological function useful for investigating sublethal effects are respiratory metabolism and locomotory activity. Both may by severely impaired by exposure to low concentrations of dispersed crude oil.

Relatively little information is available concerning the effects of crude oil on the normal activity of marine invertebrates. Hargrave and Newcombe (1973) reported an increased rate of crawling in the gastropod Littorina following exposure to seawater extracts of bunker C oil. It is possible that this is an escape response. However, several other studies indicate that in general activity is impaired rather than enhanced, following exposure of the animals to petroleum. Thus Smith (1970) found that Kuwait crude oil depressed the cirral activity of barnacles and led to a reduction in feeding and growth. Similarly, Galtsoff (1964) noted that soluble components of crude oil had an anesthetic effect upon gill cilia of certain bivalve molluscs. Exposure of the Arctic bivalve *Yoldiella intermedia* to seawater soluble components of crude oil causes the animal to cease regular feeding activity (Percy, 1974). With continued exposure the animals resumed feeding, with the duration of cessation of feeding increasing in proportion to the oil concentration.

The locomotory activity of both *Onisimus* and *Halitholus* was severely disrupted following exposure to dispersed oil. In the case of *Onisimus*, the activity inhibition may result from an anesthetic effect at lower concentrations, coupled with a physical immobilization and fouling at higher concentrations. Swimming activity of *Halitholus* is disrupted at fairly low concentrations of dispersed oil. The precise mechanism of inhibition is not yet clear. The animals cease swimming at oil levels that still permit regular pulsations of the bell to occur. However, these pulsations are either not strong enough, or insufficiently coordinated to permit the animal to swim. At the highest oil concentrations even the regular pulsations are inhibited. The inhibition generally appears to be reversible, for on transfer to clean seawater a large proportion of the animals resume swimming within 24 hours. The extent of the inhibition of activity varies considerably with oil type. Some oils appear to interfere primarily with the swimming process (Atkinson Point, Venezuela) without eliminating the associated muscular contractions while other oils (Norman Wells, Pembina) appear to disrupt swimming and also inhibit the contractile activity. The latter effect does not appear to be as reversible as the former.

The rate of respiratory metabolism is a relatively sensitive indicator of alterations in an organism's general physiological state. Only a few studies have considered the impact of crude oil on the metabolism of marine invertebrates, yet as Hargrave and Newcombe (1973) point out, metabolism can serve as a useful index of sublethal toxicity. However, the diverse metabolic responses reported in the few studies that have been carried out suggest that the interaction with the oil is complex.

Our results on the effects of crude oil on the metabolism of *Onisimus* suggest that we are dealing with a physiological response slightly more complex than the simple unidirectional responses (inhibition or enhancement of metabolism) indicated by some studies. All four of the crude oils that we have tested evoked the same basic pattern of response, although some differences in detail were evident. Low concentrations of oil-in-seawater dispersions depressed metabolism in all cases, although the magnitude of the depression varied considerably depending upon oil type. As the oil content of the dispersion increased the inhibition response was reversed and the metabolic rate either returned to the same level as that of the control (as in the case of Atkinson Point crude)

or increased to a level substantially greater than that of the controls (as in the case of the other three oils). The fact that all four oils evoked a similar pattern of reversal suggests that the effect is real.

It is possible that just such a reversal in response may account for some of the apparently contradictory results reported in the literature. In fact, in reviewing the data of Avolizi and Nuwayhid (1974) we found that a similar reversal of inhibition with increasing oil concentration is evident in the metabolism of the two bivalves *Brachidontes* and *Donax*. As the oil concentration increased through the series: 1 ml, 10 ml, 25 ml, 50 ml and 100 ml per liter, the percentage depression of metabolism of *Brachidontes* relative to that of controls varied in the sequence, 9.8%, 34.1%, 37.3%, 27.2% and 15.6%. A similar fluctuation, with the maximum depression occurring at an oil concentration of 25 ml/L was also observed for *Donax*. The authors either did not recognize the reversal of inhibition as such or attached no particular significance to it.

Further evidence in support of the phenomenon of inhibitory reversal comes from the studies on the effect of oil/corexit mixtures on metabolism. Corexit by itself depressed metabolism a maximum of about 30% and there was no evidence of a reversal of the response. In contrast, exposure to oil/Corexit mixtures depressed metabolism of low concentrations, but a reversal was once again evident as the concentration increased. Although the reversal occurred consistently with all four oil/Corexit combinations tested, the magnitude was, in all cases, much less than that observed with oil alone. It is significant that the ranking of the four oils with regard to the relative magnitude of the reversal was the same for the oil/Corexit mixtures as for the oils alone.

How is such a reversal in metabolic response with increasing oil concentration to be interpreted? A tentative scheme that is consistent with the available metabolic data and is amendable to further experimental testing is presented in Figure 44.

Oxygen utilization by an organism involves two distinct metabolic components. Basal metabolism reflects routine maintenance processes of the organism at rest. In addition, a significant portion of the oxygen uptake of many organisms is attributable to locomotory and other forms of activity.

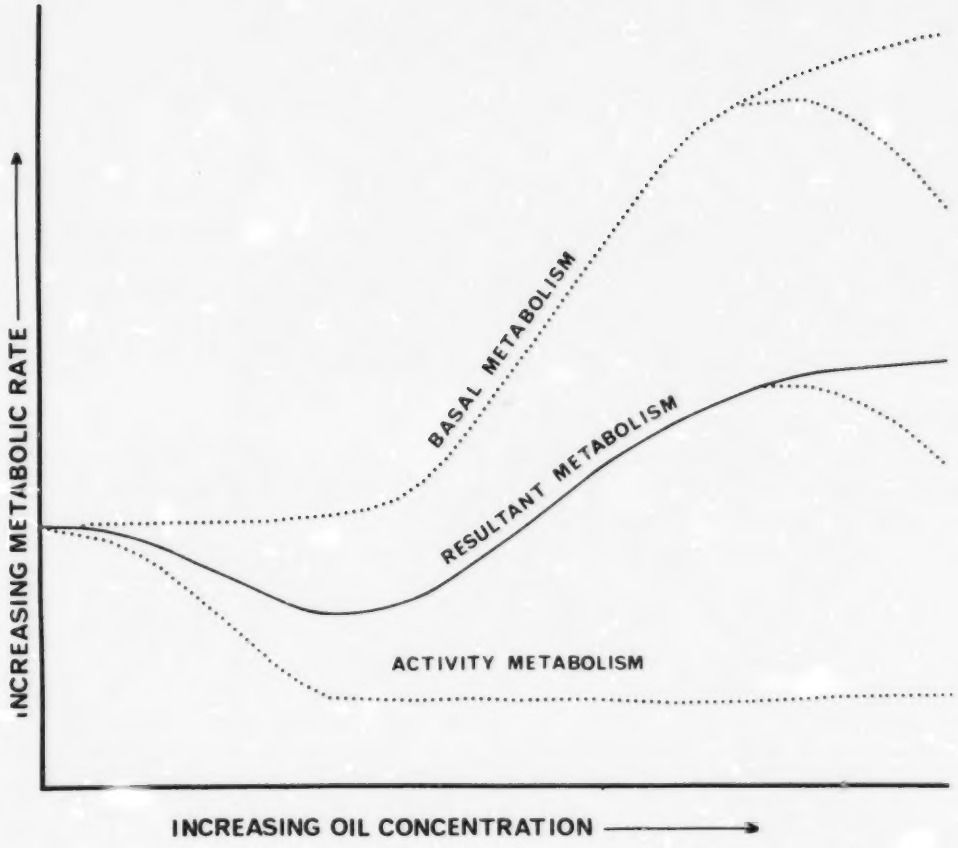

Figure 44. Hypothetical schema to explain observed metabolic responses of *Onisimus affinis* to seawater dispersions of crude oils. See text for details.

Basal and active metabolic rates in marine invertebrates have been discussed in detail by Newell (1970). Let us examine each of these two metabolic components individually in relation to the observed effects of exposure to oil dispersions.

It has already been demonstrated that the activity of *Onisimus* is severely impaired following exposure to even relatively low concentrations of dispersed crude oil. Reduction in locomotory activity in *Onisimus* is accompanied by a significant reduction in metabolic rate (Percy, 1975). Similarly, Decoursey and Vernberg (1972) noted that activity and metabolism declined in parallel following exposure to sublethal concentrations of $HgCl_2$. The metabolic consequences of an oil-induced impairment of locomotory activity is indicated by the lower dotted line in Figure 44. This curve reaches a minimum and levels out as the animal becomes completely immobile. The initial reduction in metabolism at low oil concentrations observed in *Onisimus* probably reflects such an activity effect.

It is possible to examine relative changes in cellular metabolism in the absence of activity effects by measuring the oxygen uptake of cell free homogenates. One assumption implicit in such homogenate metabolism studies is that although the absolute respiration rate of homogenized tissues is much lower than the actual in situ rate, nevertheless, the former closely reflects, in a relative way, progressive changes (such as those induced by pollution) occurring in the latter. The metabolic rate of cell free homogenates of animals pre-exposed to crude oil dispersions is elevated significantly relative to controls. There is no evidence of a depression of metabolism similar to that observed in oil exposed intact animals. The possible effect of oil exposure on basal metabolism is indicated by the upper dotted line in Figure 44. It was also observed that in animals exposed to only the seawater soluble components of the oil, the metabolic rate was significantly enhanced. Again there was no evidence of inhibition of metabolism, although there was some evidence of a decrease in the enhancement at very high concentrations. It appears that the seawater soluble components are entering the tissues and stimulating basal metabolic processes.

If the above two component metabolism curves are combined, as they would be in the intact animal, the resultant observed metabolic change with increasing oil

concentration would be similar to that indicated by the solid line in Figure 44. This curve exhibits a reversal similar to that observed experimentally. It is tentatively suggested that at low dispersion concentrations the activity is inhibited (whether by chemical or physical means is uncertain) by some component of the particulate oil, with a resulting reduction in metabolism. As the dispersion concentration increases certain of the seawater soluble components increase in concentration and penetrate the tissues where they stimulate cellular metabolism. The degree of enhancement of metabolism increases with increasing oil concentration. Above a certain concentration of the soluble components a narcosis effect comes into play and the enhancement of metabolism may be reduced. It must be emphasized that although the above scheme appears to account adequately for the observed metabolic effects it is at present very tentative and requires more intensive experimental study.

It is uncertain at present how generally applicable the above scheme might be. Our data for species other than *Onisimus* is not sufficient for comparative purposes. Metabolic depression as a result of exposure to oil dispersions is demonstrable in both *Atylus carinatus* and *Mesidotea sibirica* but it is not yet clear whether or not a consistent reversal in the response occurs with increasing oil concentration. The apparent enhancement of metabolism in *Mesidotea entomon* at all oil concentrations is unusual and requires further detailed examination.

In addition to the observations of Avolizi and Nuwayhid (1974) referred to earlier, results of a number of other metabolic studies may be pertinent in the present context. Brocksen and Bailey (1973) examined the effect of benzene (a relatively soluble component of crude oil) upon respiration of salmon and bass. They reported that the benzene was readily transported across the gills and after 24-48 hours exposure respiration increased significantly. Longer exposure resulted in a decrease in metabolism, attributed to a narcosis effect. In bass an increase in metabolism occurred on exposure to 5 ppm benzene, but at 10 ppm the respiration was depressed. Struhsaker (1974) reported essentially similar results with herring larvae exposed to benzene; at low concentrations metabolism was enhanced and at high concentrations inhibited.

Gilfillan (1972) observed that low concentrations of seawater extract of crude oil (containing primarily the water soluble components) enhanced the metabolism of the mussel *Mytilus edulis* by 20-50%.

Further evidence of the obvious complexity of the metabolic response to oil is provided by Kloth and Wohlshlag (1972) who found that a petrochemical effluent depressed metabolism of pinfish at normal environmental salinity but elevated metabolism at higher salinities. A species specific activity response may also serve to complicate the metabolic picture. Although many species decrease activity upon exposure to oil, Hargrave and Newcombe (1973) found that exposure of the winkle *Littorina* to dispersions of Bunker C oil resulted in increased activity and increased metabolic rate. On the other hand exposure to the dispersant Corexit 8666 depressed both activity and metabolism.

On the basis of the present study and information available in the literature it is clear that although the metabolic response to crude oil exposure may generally be explicable by the scheme in Figure 44, there are a variety of endogenous and exogenous factors that may have a pronounced effect on both the magnitude and direction of the resultant metabolic response. These factors include:

1) Oil type or fraction
2) Relative proportion soluble/emulsified components
3) Dose; considered as a function of both concentration and exposure time
4) Species specific activity responses
5) Concommitant environmental stresses (e.g. salinity)
6) Nutritional status

In future studies on the metabolic consequences of exposure to crude oils some consideration should be given to possible complications arising from certain of these factors.

6.2.3 Sublethal behavioral effects

Relatively few studies have been carried out on the behavioral responses of marine animals to the presence of crude oil in their environment. Evidence is accumulating,

however, that certain behavioral effects may have considerable long-term ecological significance, particularly in view of the fact that some of the adverse effects occur at very low oil concentrations. Furthermore, it is evident that the behavioral responses of marine organisms to oil in its several forms will play an important role in determining the physiologically effective dose of pollutant to which the animals will be subjected. Certain of these behavioral responses have already been briefly discussed in section 4.9. In the present study we have investigated the responses of a number of marine invertebrates to crude oil in several distinct forms in which it may be encountered in the natural environment; namely, in viscous masses or lenses, incorporated into food, and bound to bottom sediments.

Behavioral responses to the presence of oil in each of the above forms varied considerably among the different species examined. None of the animals were attracted to either oil masses or to oil-tainted sediments. The amphipod *Onisimus* and isopod *Mesidotea* were most intensively studied and each reacted to the oil in its several forms in a consistent manner. *Onisimus* tended to avoid oil masses, refused to eat oil-tainted food and were clearly repelled by oil contaminated sediments. In marked contrast, *Mesidotea* consistently exhibited a strictly neutral response to the oil; the animals appeared completely oblivious of its presence. It is interesting to note that *Mesidotea* is also extremely resistant to high concentrations of dispersed oil. In contrast, *Onisimus*, a species that is repelled by oil, is killed much more readily by oil dispersed in the water column.

Several other species although not subjected to all three of the avoidance tests did yield suggestive results in those tests that were conducted. The amphipod *Gammarus* tended to avoid crude oil, while the amphipods *Atylus* and *Corophium* were neither attracted not repelled, but exhibited an essentially neutral response to the oil.

Weathering of both the free oil and the oil-contaminated sediments markedly reduced the repellent effect upon *Onisimus*. This suggests that the animals are responding to some of the more volatile components of the oil. The avoidance response is presumably mediated by chemoreceptor organs, although the chemical compounds involved and the mechanics of the process are unknown. Pre-exposure of the animals to dispersed oil significantly reduced the avoidance response and in some cases eliminated it completely. This may represent a physiological adaptation to the oil, but more probably resulted from impairment

of chemoreceptor organs. In a similar fashion, animals exposed to heavily contaminated sediments appeared to lose the ability to detect the oil, even though at much lower concentrations they were consistently able to distinguish between tainted and untainted sediment. It is possible that with the heavily tainted sediment a significant quantity of sediment-bound oil dissolved in the overlying water where it severely impaired the animals' chemoreceptive ability. Recent work indicates that certain components of the oil may destroy the neuronal dendrites of crustacean chemoreceptor organs (Kittredge, 1973).

Both *Onisimus* and *Mesidotea* are omniverous scavangers that readily consume dead and decaying organisms. An important avenue by which such benthic species could be exposed to physiologically significant quantities of crude oil is by the consumption of oil-tainted food, in the form of fish or other organisms killed and heavily contaminated in the wake of an oil spill. A number of workers have demonstrated that fish flesh becomes tainted by exposure to even relatively low concentrations of petroleum products (Nelson-Smith, 1970; Connell, 1971).

Onisimus clearly rejected heavily tainted fish, even when presented as the only food source. It remains to be seen whether the animals would accept tainted food more readily after being starved for an extended period. At present we have no indication of the minimum degree of tainting of food sufficient to cause rejection. In contrast, *Mesidotea* readily consumed heavily tainted fish and in a choice situation show no particular preference for untainted over tainted food. Lobsters have similarly been found to consume fish very heavily contaminated with bunker C oil (Wilder, 1970). Some intertidal molluscs have been observed to ingest quantities of weathered crude oil while grazing on contaminated rocks (Smith, 1970). Certain benthic detritus feeders may also ingest large quantities of oil adsorbed onto bottom sediments.

We have as yet no very clear idea of the long-term physiological consequences of such oil ingestion. Animals exposed to crude oils may incorporate certain components into their tissues (Blumer et al., 1970). Toxic materials may in this manner be passed up the food chain, although the limited evidence available at present suggests that this does not result in concentration (magnification effect) of oil components in higher levels of the food chain (Scarratt and Zitko, 1972). Increasingly, studies are being focussed on the metabolic transformations and retention times of petroleum components in biological systems. (Fossato, 1975; Morris, 1973; Stegemen and Teal, 1973).

To understand more fully the potential ecological consequences of the behavioral interactions of marine

organisms with spilled oil we require additional information on their affinity for much lower concentrations of dispersed oil than we have dealt with here, and also on their responses to water soluble components of the oil. We particularly need to determine the threshold concentrations at which these behavioral effects occur.

6.3 Implications and potential ecological consequences

The use of physiological data, acquired under carefully controlled laboratory conditions, to explain and anticipate biological events occurring in the "real"world is a difficult enough excercise at the best of times. In the Arctic the process is rendered doubly difficult by the fact that our knowledge of conditions in the "real" world is deplorably inadequate. To be overly dogmatic under such circumstances is to be less than realistic. At best it is possible to hint at simplistic adverse effects on specific populations arising from direct encounter with the oil. We are on far less firm ground in attempting to assess the wider long-term ecological repercussions of these primary effects.

Only under very unusual circumstances will a single oil spill cause such devastation as to turn an area into a biological wasteland for a prolonged period, and even then the area involved will be very small considered on a geographic scale. Far more common are subtle modifications of biological communities arising from the differential sensitivity to the pollutant of the component populations. As this study has clearly shown Arctic marine invertebrates vary widely in their ability to tolerate crude oil. In the aftermath of a major pollution incident particularly sensitive species may be rapidly eliminated from relatively large areas. More tolerant species, confronted with less competition and/or predation may experience a virtual population explosion. Just such an effect occurred following the Buzzards Bay oil spill, when the marine worm *Capitella capitata* which was present in the area in small numbers became very abundant and flourished in all but the most heavily polluted areas (Blumer et al., 1971). Effects over wide areas would certainly be less dramatic but it is likely that given the right conditions some reduction in the diversity of species in the area would occur. This may be a particularly important consideration in the Beaufort Sea and perhaps the Arctic in general. For as Grainger (1975) points out, "the relatively simple population structure in inshore waters in particular suggests a relatively uncomplicated food chain, and this means fewer but relatively more important links in the chain and greater vulnerability on the whole than one would expect in a more complex web." At present

we know too little about Arctic marine food chains to be able to confidently pinpoint critical links that might be particularly subject to disruption by spilled oil. Intensive studies on the trophic interrelationships of arctic marine organisms are clearly required. Although we have clearly demonstrated that a variety of sublethal effects result from exposure of marine organisms to crude oil, we are still not in a position to predict, with any degree of confidence, the actual long-term ecological consequences of such physiological changes. An exposure that rapidly kills the animal is clearly significant. It is considerably more difficult to demonstrate unequivocally that a 30% reduction in metabolic rate or a 50% decrease in locomotory activity has adverse, long-term effects on a population. The effects are subtle, and the ultimate consequences are very much influenced by a wide range of inadequately known endogenous and exogenous factors. Perhaps the most critical of these is the duration of exposure to the sublethal concentrations of the pollutant. In Arctic waters, the marked reduction in the rate of weathering of the toxic components of the oil suggests that, in general, sublethal physiological perterbations would be more prolonged than after a comparable spill in temperate or tropical waters. Whether the prolongation would be sufficient to result in irreparable damage to the population is difficult to ascertain and would undoubtedly have to be considered on a species by species basis.

A further complication is introduced by the fact that a variety of behavioral and physiological dysfunctions may occur simultaneously in animals exposed to oil. Depending upon species and circumstance one or more of these adverse biological effects may assume overwhelming importance in determining the ultimate fate of the population. For example, a reluctance to burrow in oil contaminated sediments by a benthic species may result in the population being subjected to abnormally high rates of predation. If intensified predation results in the elimination of the population then the fact that the oil exposure results in a concommitant reduction in metabolic rate has little ecological significance, even though the metabolic dysfunction would itself prove fatal in the long-term. An additional, perhaps more immediately pertinent example involves the medusa *Halitholus*. It has been demonstrated that the swimming ability of these animals is rapidly impaired by exposure to relatively low concentrations of dispersed oil. This is critical for a species which must maintain its position in the uppermost layer of the water column in order to feed. A variety of other sublethal physiological effects might result from the oil exposure, but they are of little ecological consequence in

view of the fact that disruption of swimming has effectively sealed the fate of the animals. Unfortunately our knowledge is inadequate to make comparable judgements about the relative ecological importance of the various sublethal effects exhibited by the great majority of animals.

In this study we have attempted to demonstrate that with regard to oil pollution it is useful to subdivide the coastal Beaufort Sea into a number of discrete habitat types, each with its own particular faunal assemblage and each differing in the manner of intrusion of spilled oil. The quantity and dominant physical form of the oil differs in each habitat and as a consequence the nature of the principal oil/animal interactions also differ considerably. The relative proportion of the spilled oil that is incorporated into each of the habitats, and thus the nature and magnitude of the biological impact, is governed by a wide range of interacting variables that have been discussed in some detail in section 3.2.

7. CONCLUSIONS AND RECOMMENDATIONS

This study clearly illustrates the very complex nature of the potential interactions between spilled crude oil and marine ecosystems. Much of the complexity is attributable to the involvement of a wide range of interacting variables in the physical environment that to a large extent control the spread of spilled oil. The oil does not disperse uniformly through the marine environment, but intrudes in different relative quantities and in quite different physical forms into a number of distinct habitats, such as the sub-ice, the neritic and the benthic. In the near shore Beaufort Sea much of the oil will probably be concentrated in the sub-ice and benthic habitats. However, currently employed cleanup procedures may drastically alter the relative distribution of oil among these various habitats. The nature of the potentially harmful interactions between organisms and crude oil differs considerably in each of these habitats.

The problem of impact assessment is further compounded by the fact that the different species exhibit an enormous variability in response to crude oil. This variability was evident in the degree of tolerance of high concentrations of the oil, in the nature and magnitude of sublethal physiological dysfunctions resulting from exposure to oil, and finally in the sublethal behavioral responses to the presence of the oil in a variety of forms. Such variability precludes the possibility of simple generalizations concerning oil impact on animal communities. Differential sensitivity, furthermore, implies that in the aftermath of a major oil spill species diversity would be reduced in the immediate area. Selective elimination of certain sensitive species over wide areas could result in significant disruptions of the marine food web. However, the detailed knowledge required to assess the likelihood and potential magnitude of such indirect effects on trophic relationships is not yet available.

On the basis of the lethal bioassays most species examined appear to be relatively tolerant of short-term exposure to high concentrations of dispersed oil. Massive, rapid mortality among adult organisms inhabiting the neritic zone would, if it occurred at all, be limited to relatively small areas and involve only particularly sensitive species.

Species occupying sub-ice and benthic habitats may be more severely effected in the short-term than neritic species because of the tendency of oil to accumulate at these interfaces. Sub-ice organisms may be

particularly susceptible and would be subjected to smothering and fouling by viscous oil masses. In addition, the oil trapped in the sub-ice habitat weathers very slowly and retains its toxicity undiminished for extended periods. As a result, large quantities of oil could be transported considerable distances from the spill site without significant diminution of toxicity and then reintroduced into the water column. In addition, the oil would gradually accumulate and be preserved in the ice for a considerable time after initial release - and then be reintroduced into the water in large quantities at the time of spring break-up. This is also a time of initiation of intense biological activity in the surface waters of arctic seas.

Ecologically significant quantities of oil are likely to be incorporated into near shore bottom sediments as a consequence of interactions of spilled oil with the massive sediment plume of the Mackenzie River. Certain benthic species may be killed by short-term exposure to contaminated sediments, but only at oil concentrations unlikely to be realized in the natural environment except perhaps in close proximity to a spill.

In all three habitat types sublethal physiological and behavioral effects are likely to be of greater ecological significance in the long run than direct lethality, because they are induced by low oil concentrations that may occur over fairly extensive areas of the environment following a major oil spill. Both locomotory activity and respiratory metabolism of some species are severely impaired following exposure to oil. Disruption of activity may have important long-term ecological consequences by adversely effecting normal feeding or by exposing the population to increased predation.

The effects of oil on respiratory metabolism were found to be rather complex in nature. At the low concentrations of oil likely to occur widely in the water column in the wake of a major oil spill the metabolic rates of some species were depressed significantly. This appears to be related to a concommitant reduction in locomotory activity. With increasing oil concentrations a reversal of the inhibition occurs and at very high concentrations the metabolic rate may even be enhanced. A mechanism is proposed that may account for the observed metabolic effects, although the precise ecological consequences are uncertain at present.

Species differences were also evident in the behavioral responses to the presence of crude oil. Certain species appeared to be totally indifferent to the presence of the oil and readily ingested oil-tainted food and burrowed in oil contaminated sediments.

In marked contrast a number of other species exhibited a high degree of aversion to the oil; they tended to avoid oil masses, refused oil-contaminated food and overwhelmingly selected and burrowed in clean in preference to oil-contaminated sediments. The sediment preference results suggest that the animals are capable of detecting very low concentrations of sediment bound oil. These diverse behavioral responses may play an important role in determining the physiologically effective dose of oil to which specific populations may be exposed. Furthermore, the pronounced disruption of feeding and burrowing behavior noted in some species could have far reaching implication regarding the continued survival of these populations.

The potentially severe biological effects outlined in this report are likely to have relatively limited ecological significance in the wake of a single oil spill during exploratory drilling, when considered on a broad geographic scale. Far greater impact can be anticipated from the cumulative effects of continuing minor and major spills during the production and transport of oil at multiple drilling sites. In the broader ecological context the critical point will be the relative balance between the accidental input of oil on the one hand and the rate at which the oil is removed from the environment by natural processes on the other hand. Indications are that in arctic seas the rate of input required to exceed the rate of natural removal will be considerably lower than in temperate and tropical seas. In heavily exploited regions the imbalance between the two could lead to oil concentrations over wide areas approaching that at which sub-lethal physiological and behavioral effects are operative. Every effort should be made to obtain theoretical estimates of the magnitude of each of these factors under a variety of conditions. Since the only means of rigorously testing such estimates is by monitoring an actual oil spill, a detailed program should be prepared to intensively monitor the appropriate parameters in the event of an oil spill during proposed exploratory activities. Such information is essential for anticipating the potentially more serious ecological consequences of large scale production activity. In addition, further studies of sub lethal physiological and behavioral effects are required to determine the minimum concentrations of oil in the environment that are sufficient to induce significant sub-lethal dysfunction in ecologically important species. The studies presented here are suggestive, but being of limited duration and scope must be considered little more than preliminary ground breaking.

It may appear self-serving to suggest that additional expanded studies are required, but the truth of the matter is that small scale, short-term studies, no matter how carefully executed, cannot be expected to adequately fill the void resulting from years of neglect of basic arctic marine research. It is unfortunate that the pace and scope of present attempts to fill this information void are more influenced by developmental and economic considerations than by carefully considered scientific judgement.

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Table 1. Fluorimetric estimates of quantities of crude oils dispersed in seawater following standardized preparation and exposure procedures. T_p: temperature at which dispersion prepared; T_e temperature at which dispersion gently aerated in exposure chamber for 24 hours; $\bar{X}_i$: mean concentration of oil in dispersions immediately after preparation and settling ∿ corresponds to initial exposure level; $\bar{X}_f$: mean concentration of oil in dispersions after 24 hour period in exposure chambers ∿ corresponds to final exposure level; % loss: percent of oil lost from the dispersion during 24 hour exposure period.

OIL TYPE	T_p	T_e	DISPERSION TYPE	$\bar{X}_i$	N	S.D.	S.E.	$\bar{X}_f$	N	S.D.	S.E.	% LOSS
A.P.	22°C	8°C	L	18.2	8	1.864	.659	10.100	8	1.766	.624	44.5
			M	169.5	8	28.117	9.941	13.375	8	1.970	.696	92.0
			H	>800.0	8	0	0	180.500	8	33.119	11.709	
Ve.	22°C	8°C	L	16.075	8	2.149	.760	11.325	8	.828	.293	29.8
			M	21.950	8	2.822	.998	19.375	8	6.004	2.123	11.4
			H	643.000	8	102.979	36.408	93.714	7	19.059	7.204	85.7
Pe.	≈ 22°C	8°C	L	20.950	8	2.332	.824	19.375	8	4.499	1.591	7.2
			M	51.200	8	19.784	6.995	19.350	8	10.353	3.660	62.1
			H	267.750	8	22.939	8.110	72.000	8	17.599	6.222	73.1
Pe.	0°C	0°C	L	22.150	8	5.708	2.018	6.465	8	5.976	2.113	71.2
			M	63.000	8	10.810	3.822	21.850	8	14.693	5.195	65.4
			H	556.250	8	30.677	10.846	40.675	8	17.734	6.270	92.7
Pe.	≈ 20°C	8°C	L	14.625	8	7.754	2.742	1.655	8	.843	.298	88.6
			M	58.125	8	16.703	5.905	6.312	8	2.367	.837	89.0
			H	370.750	8	50.641	17.904	55.625	8	15.436	5.457	85.0

Table 1 (cont'd.)

OIL TYPE	T_p	T_e	DISPERSION TYPE	$\bar{X}_i$	N	S.D.	S.E.	$\bar{X}_f$	N	S.D.	S.E.	% LOSS
Pe.	10°C	8°C	L	8.450	8	1.696	.600	1.475	8	.388	.137	82.5
			M	50.000	8	5.555	1.964	6.925	8	2.116	.748	88.1
			H	690.000	8	75.970	26.859	25.300	8	8.093	2.861	96.3
N.W.	≈ 22°C	8°C	L	13.275	8	3.228	1.141	6.025	8	2.153	.761	54.9
			M	26.150	8	5.978	2.113	9.825	8	3.864	1.366	62.6
			H	476.250	8	85.178	30.115	34.375	8	8.943	3.162	92.8
N.W.	0°C	0°C	L	29.850	8	4.736	1.674	7.350	8	2.945	1.041	75.2
			M	74.375	8	10.084	3.565	11.900	8	2.407	.851	83.9
			H	906.250	8	75.392	26.655	29.575	8	18.284	6.464	96.7
N.W.	≈ 20°C	8°C	L	24.600	8	5.696	2.014	2.267	8	1.814	.641	90.8
			M	68.125	8	4.970	1.757	11.600	8	5.751	2.033	83.0
			H	535.000	8	14.020	4.957	60.112	8	8.590	3.037	88.8
N.W.	10°C	8°C	L	20.375	8	4.437	1.569	5.625	8	2.123	.751	72.4
			M	66.625	8	10.070	3.560	10.550	8	2.363	.836	84.2
			H	1101.250	8	133.142	47.073	54.625	8	17.087	6.041	95.0

Table 2. Mortality of *Onisimus affinis* exposed to standard dispersions of various crude oils at 8°C. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

OIL TYPE	EXPOSURE TIME (HRS)	DISPERSION TYPE			
		CONTROL	LIGHT	MEDIUM	HEAVY
Atkinson Point	24	0	0	0	0
	48	0	0	0	40
	72	0	0	0	65
	96	0	0	5	88
Venezuela	24	0	0	0	23
	48	0	0	5	90
	72	0	0	10	98
	96	0	0	18	100
Norman Wells	24	0	0	3	15
	48	0	0	3	60
	72	0	0	18	78
	96	0	0	33	95
Pembina	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	15
	96	0	0	0	25

Table 3. Mortality of *Calanus hyperboreas* exposed to standard dispersions of various crude oils at 5°C. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

OIL TYPE	EXPOSURE TIME (HRS)	DISPERSION TYPE			
		CONTROL	LIGHT	MEDIUM	HEAVY
Atkinson Point	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	10	0
Venezuela	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	0
Norman Wells	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	10
Pembina	24	0	0	10	7.5
	48	0	2.5	10	12.5
	72	2.5	12.5	15	32.5
	96	10	22.5	25	37.5

Table 4. Mortality of *Halitholus cirratus* exposed to standard dispersions of various crude oils at 8°C. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

OIL TYPE	EXPOSURE TIME (HRS)	CONTROL	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
Atkinson Point	24	10	0	0	0
	48	10	0	0	0
	72	10	0	0	0
	96	20	0	0	30
Venezuela	24	0	0	10	40
	48	0	0	10	40
	72	0	0	10	40
	96	0	0	10	100
Norman Wells	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	100
Pembina	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	80
	96	0	0	0	100

Table 5. Relative sensitivity of various arctic invertebrates to dispersions of Norman Wells crude oil at 8°C (except *C. hyperboreas* at 5°C). Results expressed as percent mortality at intervals during 96 hours exposure under standardized conditions.

SPECIES	EXPOSURE TIME (HRS)	DISPERSION TYPE CONTROL	LIGHT	MEDIUM	HEAVY
Onisimus affinis	24	0	0	3	15
	48	0	0	3	60
	72	0	0	18	78
	96	0	0	33	95
Calanus hyperboreas	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	10
Halitholus cirratus	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	100
Brachydiastylis resima	24	5	20	30	25
	48	25	35	30	25
	72	30	50	30	25
	96	45	50	30	30
Mesidotea entomon	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	0
Mesidotea sibirica	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	0
Mesidotea sabini	24	0	0	0	0
	48	0	0	0	0
	72	0	0	10	0
	96	10	0	10	0

Table 5 (cont'd.)

SPECIES	EXPOSURE TIME (HRS)	CONTROL	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
Atylus carinatus	24	0	0	0	0
	48	0	0	0	10
	72	0	0	0	15
	96	0	0	0	15
Balanus crenatus	24	0	0	0	60
	48	0	0	0	73
	72	0	0	0	73
	96	0	0	0	73
Corophium clarencense	24	0	5	2.5	0
	48	0	7.5	2.5	27.5
	72	2.5	10	7.5	45
	96	5	20	17.5	67.5
Myoxocephalus quadricornis	24	0	0	0	100
	48	0	0	0	100
	72	0	0	0	100
	96	0	0	0	100

Table 6. Mortality of *Onisimus affinis* exposed to crude oil/corexit mixtures (1:1) emulsified in seawater. Results expressed as percent mortality at intervals during 96 hours exposure under standardized conditions.

OIL TYPE	EXPOSURE TIME (HRS)	DISPERSION TYPE			
		CONTROL	LIGHT	MEDIUM	HEAVY
Corexit	24	0	0	0	0
	48	0	0	0	0
	72	0	10	0	0
	96	0	10	5	5
Venezuela Corexit	24	0	0	0	5
	48	0	0	0	45
	72	5	0	0	80
	96	5	5	10	100
Atkinson Point Corexit	24	0	0	0	0
	48	0	0	0	40
	72	0	0	0	60
	96	0	0	25	95
Norman Wells Corexit	24	0	0	0	5
	48	0	0	0	75
	72	0	0	0	90
	96	0	0	0	100
Pembina Corexit	24	0	5	0	5
	48	0	5	0	20
	72	5	10	0	95
	96	5	10	0	100

Table 7. Mortality of *Onisimus affinis* exposed to seawater dispersions of crude oils alone and in combination with corexit dispersant. Results expressed as percent mortality after 96 hours exposure at 8°C.

OIL TYPE	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
Venezuela	0	18	100
Venezuela + corexit	5	10	100
Atkinson Point	0	5	88
Atkinson Point + corexit	0	25	95
Norman Wells	0	33	95
Norman Wells + corexit	0	0	100
Pembina	0	0	25
Pembina + corexit	10	0	100

Table 8. Effect of suspended sediment on the toxicity of dispersions of Pembina crude to *Onisimus affinis* at 8°C. Results expressed as cumulative precent mortality at intervals during 96 hours exposure under standardized conditions.

	EXPOSURE TIME (HRS)	CONTROL	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
Pembina	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	15
	96	0	0	0	25
Pembina + suspended sediment	24	0	0	0	5
	48	0	0	10	15
	72	0	0	22.5	47.5
	96	2.5	5	52.5	57.5

Table 9. Effect of suspended sediment on the toxicity of dispersions of Norman Wells crude to *Onisimus affinis* at 8°C. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

	EXPOSURE TIME (HRS)	DISPERSION TYPE			
		CONTROL	LIGHT	MEDIUM	HEAVY
Norman Wells	24	0	0	0	0
	48	0	2.5	0	10
	72	2.5	5	0	17.5
	96	2.5	5	0	22.5
Norman Wells + suspended sediment	24	2.5	0	5	0
	48	7.5	2.5	10	2.5
	72	7.5	5	17.5	25
	96	10	5	25	25

Table 10. Relative sensitivity of *Onisimus affinis* to dispersions of Norman Wells crude oil at different times of the year. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

	EXPOSURE TIME (HRS)	CONTROL	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
July 25	24	0	0	2.5	15
	48	0	0	2.5	60
	72	0	0	17.5	77.5
	96	0	0	32.5	95
May 5	24	0	0	0	0
	48	0	2.5	0	10
	72	2.5	5	0	17.5
	96	2.5	5	0	22.5
March 17	24	0	2.5	0	0
	48	0	2.5	0	0
	72	5	2.5	0	17.5
	96	7.5	5	5	42.5

Table 11. Relative sensitivity of *Onisimus affinis* to dispersions of Pembina crude oil at different times of the year. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions.

	EXPOSURE TIME (HRS)	CONTROL	DISPERSION TYPE LIGHT	MEDIUM	HEAVY
August 21	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	15
	96	0	0	0	25
April 7	24	5	0	0	0
	48	5	0	0	2.5
	72	5	0	0	7.5
	96	5	0	2.5	27.5

Table 12. Effect of temperature on the toxicity of seawater dispersions of Pembina crude oil to *Onisimus affinis*. Results expressed as cumulative percent mortality at intervals during 96 hours exposure under standardized conditions. T_p: temperature at which dispersion prepared; T_e: temperature of dispersion during exposure of animals.

T_p(°C)	T_e(°C)	EXPOSURE TIME (HRS)	DISPERSION TYPE CONTROL	LIGHT	MEDIUM	HEAVY
22°	8°	24	5.0	0	0	0
		48	5.0	0	0	2.5
		72	5.0	0	0	7.5
		96	5.0	0	2.5	27.5
22°	0°	24	0	0	0	0
		48	0	0	0	0
		72	0	2.5	0	0
		96	0	2.5	0	0
0°	8°	24	0	0	0	0
		48	0	0	0	2.5
		72	0	0	2.5	20.0
		96	0	0	10	35.0
0°	0°	24	0	0	2.5	0
		48	0	0	2.5	0
		72	0	2.5	2.5	0
		96	0	2.5	2.5	2.5

Table 13. Effect of temperature on the toxicity of seawater dispersions of Norman Wells crude oil to *Onisimus affinis*. Results expressed as cumulative precent mortality at intervals during 96 hours exposure under standardized conditions. T_p: temperature at which dispersion prepared; T_e: temperature of dispersion during exposure of animals. Sub-ice oil sample recovered in May, 1975 from controlled under-ice spill in November, 1974. See text for details.

	T_p(°C)	T_e(°C)	EXPOSURE TIME (HRS)	CONTROL	LIGHT	MEDIUM	HEAVY
Fresh oil	22°	8°	24	0	2.5	0	0
			48	0	2.5	0	0
			72	7.5	2.5	0	17.5
			96	7.5	5.0	5.0	42.5
	22°	0°	24	0	0	0	0
			48	0	0	0	0
			72	0	0	2.5	5.0
			96	0	0	2.5	7.5
	0°	8°	24	0	0	2.5	12.5
			48	0	0	2.5	32.5
			72	0	0	2.5	50.0
			96	0	0	7.5	75.0
	0°	0°	24	2.5	5.0	2.5	2.5
			48	2.5	7.5	10.0	10.0
			72	2.5	7.5	10.0	60.0
			96	2.5	7.5	10.0	60.0
Sub-ice oil sample	0°	0°	24	0	0	0	0
			48	0	0	0	12.5
			72	0	0	0	32.5
			96	0	0	2.5	47.5

Table 14. Toxicity of oil-tainted sediments to *Onisimus affinis* at 0°C. Results expressed as percent mortality during 10 day exposure to sediment. Approximately one week elapsed between termination of exposure for one group and initiation of exposure to the same sediment of subsequent fresh group of animals. Twenty animals exposed to each oil during each exposure period.

OIL TYPE	LEVEL	EXPOSURE PERIOD 1	EXPOSURE PERIOD 2	EXPOSURE PERIOD 3	EXPOSURE PERIOD 4
Control		10.0	7.5	22.5	5.0
N.W.	Medium	32.5	5.0	5.0	5.0
N.W.	Heavy	100.0	65.0	82.5	52.5
Pe.	Medium	15.0	7.5	5.0	10.0
Pe.	Heavy	97.5	52.5	45.0	22.5
A.P.	Medium	0	0	0	7.5
A.P.	Heavy	62.5	12.5	7.5	10.0

Table 15. Toxicity of oil-tainted sediments to *Onisimus affinis* at 10°C. Results expressed as percent mortality during ten day exposure to sediment. Approximately one week elapsed between termination of exposure for one group and initiation of exposure to the same sediment of subsequent fresh group of animals. Twenty animals exposed to each oil during each exposure period.

OIL TYPE	LEVEL	EXPOSURE PERIOD 1	EXPOSURE PERIOD 2	EXPOSURE PERIOD 3	EXPOSURE PERIOD 4
Control		0	5.0	0	5.0
N.W.	Medium	2.5	0	2.5	0
N.W.	Heavy	87.5	87.5	25.0	25.0
Pe.	Medium	10.0	10.0	7.5	2.5
Pe.	Heavy	57.5	67.5	15.0	5.0
A.P.	Medium	2.5	2.5	0	2.5
A.P.	Heavy	60.0	15.0	15.0	2.5

Table 16. Effect of 24 hour pre-exposure to seawater dispersions of various crude oils on the respiratory metabolism of *Onisimus affinis* at 8°C. Mean respiration rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr. Significance of changes from the control rate determined by Student's t test.

OIL TYPE	DISPERSION TYPE	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	Δ%	t	p
	Control	37	0.214	0.057	0.009	0.400	0.110	-	-	-
N.W.	Light	8	0.170	0.035	0.013	0.220	0.110	-20.6	2.07	<0.05
N.W.	Medium	8	0.222	0.036	0.013	0.280	0.170	+ 3.7	-0.38	NS
N.W.	Heavy	8	0.251	0.052	0.018	0.340	0.190	+17.3	-1.67	<0.10
A.P.	Light	8	0.167	0.042	0.015	0.250	0.120	-22.0	2.17	<0.05
A.P.	Medium	8	0.160	0.041	0.014	0.220	0.120	-25.2	2.50	<0.01
A.P.	Heavy	8	0.212	0.055	0.020	0.280	0.150	- 0.9	0.09	NS
Ve.	Light	8	0.199	0.077	0.027	0.340	0.080	- 7.0	0.62	NS
Ve.	Medium	8	0.187	0.021	0.007	0.210	0.150	-12.6	1.30	<0.1
Ve.	Heavy	8	0.249	9.035	0.012	0.310	0.200	+16.4	1.64	<0.1
Pe.	Light	8	0.177	0.024	0.008	0.220	0.140	-17.3	1.77	<0.05
Pe.	Medium	8	0.172	0.021	0.007	0.210	0.150	-19.6	2.03	<0.025
Pe.	Heavy	8	0.301	0.041	0.014	0.360	0.230	+40.7	4.03	<0.005

Table 17. Effect of exposure for varying periods to seawater dispersions (light) of Norman Wells crude oil on the respiratory metabolism of *Onisimus affinis* at 8°C. Mean respiration rate ($\bar{x}$) expressed as µl O_2/mg dry wt/hr. Animals fed after 96 hours exposure. Significance of changes from control rate determined by Student's t test.

	EXPOSURE TIME										
	0 hour	24 hours		48 hours		72 hours		96 hours		120 hours	
	Control	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
n	14	10	11	11	11	10	11	8	11	10	11
$\bar{x}$	0.430	0.308	0.265	0.276	0.250	0.251	0.242	0.260	0.224	0.384	0.272
S.D.	0.077	0.045	0.029	0.063	0.064	0.031	0.032	0.049	0.054	0.066	0.066
S.E.	0.021	0.014	0.009	0.019	0.019	0.010	0.010	0.017	0.016	0.021	0.020
Max.	0.604	0.368	0.323	0.394	0.363	0.302	0.285	0.354	0.310	0.456	0.392
Min.	0.333	0.222	0.207	0.201	0.188	0.207	0.195	0.192	0.118	0.274	0.181
Δ%		-14.0		-9.4		-3.6		-13.8		-29.2	
t		2.53		0.91		1.62		1.41		3.68	
p		<0.025		<0.20		<0.10		<0.10		<0.005	

Table 18. Effect of 24 hour exposure to seawater dispersions of crude oils on the respiratory metabolism of *Mesidotea sibirica* and *Mesidotea entomon* at 8°C. Mean respiratory rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr.

DISPERSION TYPE	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	Δ%	t	p
Mesidotea sibirica									
Control	9	0.148	0.021	0.007	0.189	0.125	-	-	-
N.W. Light	9	0.149	0.028	0.009	0.185	0.102	+ 0.7	-0.08	<0.50
N.W. Medium	9	0.097	0.023	0.008	0.144	0.062	-34.5	4.62	<0.005
N.W. Heavy	9	0.144	0.048	0.016	0.234	0.105	- 2.7	0.22	<0.025
Mesidotea sibirica									
Control	9	0.147	0.043	0.014	0.215	0.072	-	-	-
Pe. Light	9	0.102	0.034	0.011	0.160	0.060	-30.6	2.28	<0.025
Pe. Medium	9	0.120	0.034	0.011	0.200	0.080	-18.4	1.37	<0.10
Pe. Heavy	9	0.112	0.023	0.008	0.140	0.080	-23.8	2.02	<0.05
Mesidotea entomn									
Control	8	0.159	0.044	0.016	0.220	0.090	-	-	-
N.W. Light	7	0.223	0.091	0.034	0.370	0.120	+40.3	-1.65	<0.10
N.W. Medium	8	0.191	0.080	0.028	0.340	0.100	+20.1	-0.93	<0.20
N.W. Heavy	9	0.266	0.075	0.025	0.370	0.150	+67.3	-3.33	<0.005

Table 19. Effect of 24 hour exposure to seawater dispersions of crude oils on the respiratory metabolism of *Atylus carinatus* at 8°C. Mean respiratory rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr.

DISPERSION TYPE	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	Δ%	t	p
Control	9	0.562	0.098	0.033	0.690	0.410	-	-	-
N.W. Light	9	0.570	0.096	0.032	0.740	0.400	+1.4	-0.17	<0.45
N.W. Medium	9	0.521	0.075	0.025	0.670	0.410	-7.3	0.94	<0.20
N.W. Heavy	9	0.546	0.105	0.035	0.750	0.380	-2.8	0.32	<0.40
Control	9	0.581	0.093	0.031	0.680	0.400	-	-	-
Pe. Light	9	0.541	0.081	0.027	0.690	0.460	-6.9	0.91	<0.20
Pe. Medium	9	0.457	0.075	0.025	0.590	0.340	-21.3	2.93	<0.005
Pe. Heavy	9	0.432	0.123	0.041	0.610	0.280	-25.6	2.72	<0.01

Table 20. Effect of 24 hour exposure to seawater dispersions of various crude oils in combination with the dispersant Corexit (1:1 v/v) on the respiratory metabolism of *Onisimus affinis* at 8°C. Mean respiration rate $\bar{x}$ expressed as µl O_2/mg dry weight/hr. Significance of changes from the control rate determined by Student's t test.

OIL TYPE	DISPERSION TYPE	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	Δ%	t	p
	Control	16	0.304	0.069	0.017	0.430	0.223	-	-	-
Corexit	Light	8	0.290	0.060	0.021	0.363	0.218	- 4.6	0.47	NS
Corexit	Medium	8	0.214	0.039	0.014	0.274	0.155	-29.6	3.27	<0.005
Corexit	Heavy	8	0.219	0.040	0.014	0.289	0.154	-28.0	3.08	<0.005
A.P. + C	Light	8	0.200	0.066	0.023	0.343	0.148	-34.2	3.37	<0.005
A.P. + C	Medium	8	0.202	0.034	0.012	0.248	0.144	-33.6	3.76	<0.005
A.P. + C	Heavy	8	0.270	0.018	0.007	0.291	0.245	-11.2	1.31	NS
N.W. + C	Light	8	0.245	0.040	0.014	0.310	0.200	-19.4	2.14	<0.025
N.W. + C	Medium	8	0.233	0.042	0.015	0.312	0.186	-23.4	2.54	<0.01
N.W. + C	Heavy	8	0.298	0.060	0.021	0.358	0.187	- 2.0	0.20	NS
	Control	16	0.367	0.051	0.017	0.470	0.290	-	-	-
Pe. + C	Light	9	0.384	0.087	0.029	0.530	0.270	+ 4.6	0.59	NS
Pe. + C	Medium	9	0.273	0.050	0.017	0.360	0.220	-25.6	4.27	<0.005
Pe. + C	Heavy	9	0.441	0.067	0.022	0.540	0.340	+20.2	2.97	<0.005

Table 21. Effect of short-term exposure to various concentrations of seawater soluble components of Norman Wells crude oil on the respiratory metabolism of *Onisimus affinis* at 7°C[1]. Mean respiration rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr. Significance of changes from the control rate determined by Student's t test.

RELATIVE OIL CONC.	$\bar{x}$	N	S.D.	S.E.	Δ%	t	P
0	0.340	10	0.115	0.036	-	-	-
10	0.352	13	0.066	0.018	+3.5	-0.30	N.S.
100	0.354	13	0.094	0.026	+4.1	-0.30	N.S.
1,000	0.473	14	0.064	0.017	+25.0	-3.50	<0.005
5,000	0.469	13	0.035	0.010	+38.0	-3.69	<0.005
10,000	0.376	13	0.034	0.009	+10.6	-1.03	N.S.

[1](Table from Percy, 1974)

Table 22. Effect of short-term exposure to various concentrations of seawater soluble components of Atkinson Point crude oil on the respiratory metabolism of *Onisimus affinis* at 7°C[1]. Mean respiration rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr. Significance of changes from the control rate determined by Student's t test.

RELATIVE OIL CONC.	$\bar{x}$	N	S.D.	S.E.	Δ%	t	P
0	0.340	10	0.115	0.036	-	-	-
10	0.409	13	0.114	0.032	+20.3	-1.37	N.S.
100	0.369	13	0.109	0.030	+8.5	-0.59	N.S.
1,000	0.425	16	0.041	0.035	+25.0	-1.44	N.S.
5,000	0.443	17	0.146	0.035	+30.3	-1.83	<0.05
10,000	0.426	22	0.118	0.025	+25.3	-1.82	<0.05

[1](Table from Percy, 1974)

Table 23. Effect of 24 hour exposure to seawater dispersions of Norman Wells crude oil on the respiratory metabolism of cell-free homogenates of *Onisimus affinis* at 14°C. Mean respiration rate ($\bar{x}$) expressed as µl O_2/mg dry weight/hr.

DISPERSION TYPE	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	Δ%	t	P
Control	9	0.133	0.031	0.010	0.180	0.090	-	-	-
N.W. Light	7	0.189	0.029	0.011	0.240	0.160	+42.1	-3.44	<0.005
N.W. Medium	8	0.147	0.025	0.009	0.190	0.110	+10.5	-0.95	<0.20
N.W. Heavy	7	0.194	0.037	0.014	0.230	0.120	+45.9	-3.36	<0.005

Table 24. Assessment of variability of locomotory activity of *Onisimus affinis* as determined by the annular chamber technique. Mean activity ($\bar{x}$) expressed as line crossings/minute. All measurements made on the same day at 8°C.

GROUP	N	$\bar{x}$	S.D.	S.E.	MAX.	MIN.	V[1]
A	10	57.1	12.5	3.9	77.8	35.3	21.9
B	10	76.7	16.8	5.3	113.8	56.3	21.9
C	10	50.9	14.5	4.6	72.8	15.8	28.5
D	10	69.4	15.5	4.9	107.0	49.8	22.3

Coefficient of variation $V = \frac{S.D.}{\bar{x}} \times 100$

Table 25. Effect of 24 hour exposure to seawater dispersions of various crude oils on the locomotory activity of *Onisimus affinis* at 8°C. Mean activity ($\bar{x}$) expressed as line crossings/minute.

DISPERSION TYPE	N	NO. DEAD	$\bar{X}$	S.D.	S.E.	MAX.	MIN.	Δ%
Control	10	0	44.8	14.5	4.6	74.7	15.5	-
N.W. Light	10	0	24.6	14.2	4.5	53.5	6.0	55.5
N.W. Medium	10	1	1.7	3.0	0.9	9.2	0.0	3.9
N.W. Heavy	10	4	1.1	2.3	0.7	6.0	0.0	2.6
Control	9	0	32.5	21.9	7.3	65.2	2.2	-
Ve. Light	10	0	20.0	14.9	4.7	56.2	2.7	61.7
Ve. Medium	10	0	7.2	5.2	1.6	16.5	0.0	22.3
Ve. Heavy	10	0	0.8	1.3	0.4	3.0	0.0	3.0
Control	10	0	39.4	18.5	5.8	77.0	21.5	-
Pe. Light	10	0	21.4	7.3	2.3	31.0	9.5	54.2
Pe. Medium	10	0	7.6	8.0	2.5	23.0	0.0	19.2
Pe. Heavy	10	0	0.9	1.5	0.5	4.5	0.0	2.2
AFTER 24 HR RECOVERY								
Control	10	0	41.2	18.9	6.0	66.5	2.0	-
Pe. Light	10	0	20.1	11.1	3.5	38.5	0.0	48.8
Pe. Medium	10	0	12.3	11.9	3.8	31.0	0.0	29.7
Pe. Heavy	10	2	1.0	1.5	0.5	4.0	0.0	2.4

Table 26. Assessment of variability of pulsation rate of *Halitholus cirratus*. All determinations made at 7°- 8°C. Mean activity ($\bar{X}$) expressed as contractions per minute. V is the coefficient of variation as in Table 24.

DATE	GROUP	N	$\bar{X}$	S.D.	S.E.	MAX.	MIN.	V
18/6/75	A	10	39.6	7.0	2.2	50.0	28.0	17.7
	B	10	40.3	7.1	2.3	50.0	27.0	17.6
	C	10	39.2	9.3	2.9	54.5	27.5	23.7
	D	10	43.0	7.1	2.2	53.0	31.0	16.5
27/6/75	A	10	45.5	4.7	1.5	51.0	37.0	10.3
	B	10	41.4	7.3	2.3	53.5	31.5	17.6
	C	10	42.9	6.7	2.1	54.0	30.5	15.6
	D	10	37.0	6.5	2.1	47.0	27.5	17.6

Table 27. Effect of 24 hour exposure to seawater dispersions of Norman Wells crude oil on the activity of *Halitholus cirratus* at 8°C. Pre-exposure activity determined immediately before placing animals in exposure tanks, post-exposure activity determined after 24 hours exposure and recovery activity measured 24 hours after return to clean seawater. Mean pulsation rate (bpm) determined only for animals swimming or actively pulsating. Refer to text for method of determination of activity score.

DISPERSION TYPE	EXPOSURE	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	MEAN bpm	ACTIVITY SCORE (%)
Control	Pre -	10	0	0	53	100
	Post -	10	0	0	47	100
	Recovery	10	0	0	45	100
Light	Pre -	10	0	0	48	100
	Post -	4	3	3	44	55
	Recovery	10	0	0	36	100
Medium	Pre -	10	0	0	53	100
	Post -	3	3	4	50	45
	Recovery	10	0	0	42	100
Heavy	Pre -	10	0	0	56	100
	Post -	0	0	10	-	0
	Recovery	0	6	4	28	30

Table 28. Effect of 24 hour exposure to seawater dispersions of Pembina crude oil on the activity of *Halitholus cirratus* at 8°C. Refer to Table 27 for explanation of table.

DISPERSION TYPE	EXPOSURE	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	MEAN bpm	ACTIVITY SCORE (%)
Control	Pre -	10	0	0	40	100
	Post -	9	0	1	35	90
Light	Pre -	10	0	0	40	100
	Post -	3	7	0	-	65
Medium	Pre -	10	0	0	39	100
	Post -	0	2	8	-	10
Heavy	Pre -	10	0	0	43	100
	Post -	0	0	10	-	0

Table 29. Effect of 24 hour exposure to seawater dispersions of Atkinson Point crude oil on the activity of *Halitholus cirratus* at 8°C. Refer to Table 27 for explanation of Table.

DISPERSION TYPE	EXPOSURE	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	MEAN bpm	ACTIVITY SCORE (%)
Control	Pre -	10	0	0	46	100
	Post -	10	0	0	55	100
	Recovery	8	2	0	50	90
Light	Pre -	10	0	0	41	100
	Post -	10	0	0	42	100
	Recovery	10	0	0	47	100
Medium	Pre -	10	0	0	43	100
	Post -	10	0	0	46	100
	Recovery	6	3	1	33	75
Heavy	Pre -	10	0	0	37	100
	Post -	9	1	0	37	95
	Recovery	6	3	1	41	75

Table 30. Effect of 24 hour exposure to seawater dispersions of Venezuela crude oil on the activity of *Halitholus cirratus* at 8°C. Refer to Table 27 for explanation of Table.

DISPERSION TYPE	EXPOSURE	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	MEAN bpm	ACTIVITY SCORE (%)
Control	Pre -	10	0	0	47	100
	Post -	10	0	0	40	100
	Recovery	10	0	0	46	100
Light	Pre -	10	0	0	49	100
	Post -	8	0	2	33	80
	Recovery	10	0	0	48	100
Medium	Pre -	10	0	0	47	100
	Post -	10	0	0	41	100
	Recovery	8	2	0	38	85
Heavy	Pre -	10	0	0	50	100
	Post -	0	10	0	44	50
	Recovery	4	6	0	32	70

Table 31. Effect of short-term exposure to seawater dispersions (medium) of Venezuela crude oil on the activity of *Halitholus cirratus* at 8°C. Refer to text for method of determination of activity score. Control and oil exposed groups run simultaneously.

EXPOSURE	EXPOSURE TIME (HRS)	N	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	ACTIVITY SCORE (%)
Control	0	10	10	0	0	100
	1	10	10	0	0	100
	2	10	10	0	0	100
	3	10	10	0	0	100
	4	10	10	0	0	100
	5	10	9	1	0	95
	6	10	7	3	0	85
Ve. Medium	0	10	10	0	0	100
	1	10	1	9	0	55
	2	10	0	10	0	50
	3	10	0	9	1	45
	4	10	0	9	1	45
	5	10	0	9	1	45
	6	10	0	9	1	45

Table 32. Effect of short-term exposure to seawater dispersions (medium) of Norman Wells crude oil on the activity of *Halitholus cirratus* at 8°C. Control and oil exposed groups run simultaneously.

EXPOSURE	EXPOSURE TIME (HRS)	N	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	ACTIVITY SCORE (%)
Control	0	10	10	0	0	100
	1	10	8	2	0	90
	2	10	10	0	0	100
	3	10	10	0	0	100
	4	10	9	1	0	95
	5	10	9	1	0	95
	6	10	10	0	0	100
N.W.Medium	0	10	10	0	0	100
	1	10	1	9	0	50
	2	10	0	9	1	45
	3	10	0	5	5	25
	4	10	0	6	4	30
	5	10	0	6	4	30
	6	10	0	4	6	20

Table 33. Effect of short-term exposure to seawater dispersions (medium) of Pembina crude oil on the activity of *Halitholus cirratus* at 8°C. Control and oil exposed groups run simultaneously

EXPOSURE	EXPOSURE TIME (HRS)	N	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	ACTIVITY SCORE (%)
Control	0	10	9	0	1	90
	1	10	10	0	0	100
	2	10	8	2	0	90
	3	10	10	0	0	100
	4	10	10	0	0	100
	5	10	9	1	0	95
	6	10	10	0	0	100
	Recovery[1]	10	10	0	0	100
Pe. Medium	0	10	10	0	0	100
	1	10	4	3	3	55
	2	10	4	5	1	65
	3	10	1	5	4	35
	4	10	1	3	6	25
	5	10	2	2	7	20
	6	10	1	3	6	25
	Recovery[1]	10	2	5	3	45

[1]Animals returned to clean seawater and activity determined after 24 hours.

Table 34. Effect of short-term exposure to seawater dispersions (medium) of Atkinson Point crude oil on the activity of *Halitholus cirratus* at 8°C. Control and oil exposed groups run simultaneously.

EXPOSURE	EXPOSURE TIME (HRS)	N	NUMBER SWIMMING	NUMBER PULSATING	NUMBER QUIESCENT	ACTIVITY SCORE (%)
Control	0	10	10	0	0	100
	1	10	6	4	0	85
	2	10	9	0	1	90
	3	10	9	0	1	90
	4	10	7	1	2	75
	5	10	7	3	0	85
	6	10	7	1	2	75
A.P. Medium	0	10	10	0	0	100
	1	10	6	4	0	80
	2	10	1	9	0	55
	3	10	0	10	0	50
	4	10	0	10	0	50
	5	10	0	10	0	50
	6	10	0	10	0	50

Table 35. Behavioral responses of *Gammarus oceanicus*, *Mesidotea entomon* and *Onisimus affinis* in the presence of Atkinson Point, Norman Wells and Venezuela crude oils. Attraction-repulsion response expressed as affinity coefficient (A.C.). Other symbols as described in section 4.9.1.

SPECIES	OIL TYPE	$\Sigma O'_z$	$\Sigma e'_z$	A.C.	X^2	P
Gammarus	A.P.	171	260	-34.2	45.8	<0.005
	N.W.	214	260	-17.7	16.3	<0.005
	Ve.	212	257	-17.5	13.0	<0.05
Mesidotea	A.P.	256	265	-3.3	0.5	N.S.
	N.W.	206	248	-16.9	10.4	<0.05
	Ve.	265	271	-2.2	1.0	N.S.
Onisimus	A.P.	176	293	-39.9	103.6	<0.005
	N.W.	199	320	-37.8	90.2	<0.005
	Ve.	228	300	-23.9	30.8	<0.005
Onisimus (weathered oil)						
	A.P.	352	331	+6.5	2.6	N.S.
	N.W.	217	328	-33.9	75.1	<0.005
	Ve.	321	327	-1.7	0.2	N.S.
Onisimus (pre-exposed)						
	A.P.	334	378	-11.7	10.2	<0.05
	N.W.	265	268	-1.2	0.1	N.S.
	Ve.	295	317	-6.9	3.1	N.S.

Table 36. Behavioral response of *Onisimus affinis* to sediments freshly contaminated with crude oils (Series A).

OIL TYPE	OIL CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	94	50	95.7	4.3	39.51	<0.005
	Medium	99	17	94.1	5.9	13.24	<0.005
	Heavy	98	17	76.5	23.5	4.90	<0.05
	Heavy +	101	26	53.9	46.1	0.16	N.S.
Venezuela	Light	100	51	96.1	3.9	43.31	<0.005
	Medium	102	59	95.0	5.0	48.60	<0.005
	Heavy	101	12	83.3	16.7	5.33	<0.05
	Heavy +	102	17	64.7	35.3	1.49	N.S.
Pembina	Light	101	38	100.0	0.0	38.00	<0.005
	Medium	109	29	90.6	9.4	21.26	<0.005
	Heavy	98	6	100.0	0.0	6.00	<0.025
	Heavy +	100	20	55.0	45.0	0.20	N.S.
Atkinson Point	Light	104	38	92.5	7.5	31.90	<0.005
	Medium	109	57	83.9	16.1	36.28	<0.005
	Heavy	102	24	87.5	12.5	13.50	<0.005
	Heavy +	103	29	70.0	30.0	4.80	<0.05

Table 37. Behavioral response of *Onisimus affinis* to sediments freshly contaminated with crude oils (series B)

OIL TYPE	OIL CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	101	42	95.2	4.8	34.38	<0.005
	Medium	96	34	90.9	9.1	22.09	<0.005
	Heavy	97	12	50.0	50.0	0.00	N.S.
	Heavy +	94	32	30.0	70.0	4.80	<0.05
Pembina	Light	96	44	90.5	9.5	27.52	<0.005
	Medium	95	38	91.7	8.3	25.00	<0.005
	Heavy	105	14	93.3	6.7	11.26	<0.005
	Heavy +	95	14	61.5	38.5	0.69	N.S.
Atkinson Point	Light	93	58	87.0	13.0	29.63	<0.005
	Medium	110	48	88.7	11.3	31.72	<0.005
	Heavy	96	47	95.6	4.4	37.36	<0.005
	Heavy +	99	31	87.1	12.9	17.06	<0.005

Table 38. Behavioral response of *Onisimus affinis* to sediments contaminated with crude oil and then permitted to weather for 1 week in circulating seawater system.

OIL TYPE	CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	105	55	41.4	58.6	1.72	N.S.
	Medium	99	55	90.7	9.3	35.85	<0.005
	Heavy	97	34	93.9	6.1	25.49	<0.005
	Heavy +	95	27	69.2	30.8	3.85	<0.05
Venezuela	Light	104	58	25.0	75.0	15.00	<0.005
	Medium	114	67	71.1	28.9	6.74	<0.01
	Heavy	101	60	78.7	21.3	20.08	<0.005
	Heavy +	100	61	75.4	24.6	15.75	<0.005
Pembina	Light	98	45	72.7	27.3	20.00	<0.005
	Medium	104	53	65.4	34.6	5.26	<0.05
	Heavy	100	43	74.4	25.6	10.26	<0.01
	Heavy +	102	30	71.0	29.0	5.45	<0.02
Atkinson Point	Light	96	55	54.7	45.3	0.47	N.S.
	Medium	96	50	54.2	45.8	0.33	N.S.
	Heavy	98	66	80.0	20.0	23.40	<0.005
	Heavy +	92	59	75.9	24.1	14.52	<0.005

Table 39. Behavioral response of *Mesidotea entomon* to sediments freshly contaminated with crude oil

OIL TYPE	CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	20	35	42.9	57.1	0.14	N.S.
	Medium	20	45	55.6	44.4	0.11	N.S.
	Heavy	20	45	66.7	33.3	1.00	N.S.
	Heavy +	20	40	37.5	62.5	0.50	N.S.
Pembina	Light	20	60	41.7	58.3	0.33	N.S.
	Medium	20	65	69.2	30.8	1.92	N.S.
	Heavy	20	35	0.0	100.0	7.00	<0.01
	Heavy +	20	60	66.7	33.3	1.33	N.S.
Atkinson Point	Light	20	30	33.3	66.7	0.66	N.S.
	Medium	20	70	50.0	50.0	0.00	N.S.
	Heavy	20	60	50.0	50.0	0.00	N.S.
	Heavy +	20	35	85.7	14.3	3.57	N.S.

Table 40. Behavioral response of *Mesidotea sibirica* to sediments freshly contaminated with crude oils.

OIL TYPE	CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	20	55	63.6	36.4	0.84	N.S.
	Medium	20	40	75.0	25.0	2.06	N.S.
	Heavy	20	30	83.3	16.7	2.67	N.S.
	Heavy+	20	50	60.0	40.0	0.43	N.S.
Venezuela	Light	20	55	72.7	27.3	2.32	N.S.
	Medium	20	45	55.6	44.4	0.11	N.S.
	Heavy	20	35	57.1	42.9	0.18	N.S.
	Heavy +	20	65	53.8	46.2	0.09	N.S.
Pembina	Light	20	55	45.5	54.5	0.10	N.S.
	Medium	20	40	50.0	50.0	0.00	N.S.
	Heavy	20	65	30.8	69.2	1.92	N.S.
	Heavy +	20	30	33.3	66.7	0.67	N.S.
Atkinson Point	Light	20	45	44.4	55.6	0.11	N.S.
	Medium	20	60	41.7	58.3	0.33	N.S.
	Heavy	20	45	66.7	33.3	1.00	N.S.
	Heavy +	20	55	45.5	54.5	0.09	N.S.

Table 41. Behavioral response of *Corophium clarencense* to sediments freshly contaminated with crude oils

OIL TYPE	OIL CONCENTRATION	N	% IN SEDIMENT	RELATIVE DISTRIBUTION (%) CONTROL	OILED	X^2	P
Norman Wells	Light	72	38	51.9	48.1	0.04	N.S.
	Medium	39	90	22.9	77.1	10.38	<0.005
	Heavy	67	69	78.3	21.7	14.83	<0.005
	Heavy +	32	91	37.9	62.1	1.70	N.S.
Venezuela	Light	62	27	64.7	35.3	1.49	N.S.
	Medium	64	30	63.2	36.8	1.35	N.S.
	Heavy	71	27	47.4	52.6	0.06	N.S.
	Heavy +	61	18	27.3	72.7	2.32	N.S.
Pembina	Light	60	20	66.7	33.3	1.33	N.S.
	Medium	57	33	52.6	47.4	0.06	N.S.
	Heavy	61	30	50.0	50.0	0.00	N.S.
	Heavy +	59	25	46.7	53.3	0.67	N.S.
Atkinson Point	Light	63	33	66.7	33.3	2.33	N.S.
	Medium	59	20	75.0	25.0	3.00	N.S.
	Heavy	60	28	70.6	29.4	2.92	N.S.
	Heavy +	60	25	33.3	66.7	1.67	N.S.

Table 42. Behavioral response of *Onisimus affinis* and *Mesidotea entomon* presented with oil-tainted (O+) and untainted (O-) fish squares simultaneously. Each oil tested in triplicate (a, b & c) using different animals each time.

OIL TYPE	a		b		c					
	O+	O-	O+	O-	O+	O-	ΣO+	ΣO-	%O+	%O-
Onisimus										
A. P.	3	8	17	73	1	31	21	112	15.8%	84.2%
N. W.	6	34	6	82	23	28	35	144	19.6%	80.4%
Ve.	0	124	2	125	0	82	2	331	0.6%	99.4%
Mesidotea										
N.W.	37	24	42	21	10	33	89	78	53.3%	46.7%

Table 43. Behavioral response of *Onisimus affinis* and *Mesidotea entomon* to food tainted with various crude oils. Results expressed as percent feeding activity as described in text.

SPECIES	OIL TYPE	CONTROL	PERCENT FEEDING ACTIVITY			MEAN
			a	b	c	
Onisimus	Unoiled	-	67%	87%	87%	80%
Onisimus	A.P.	64%	3%	6%	2%	4%
Onisimus	N.W.	45%	5%	3%	8%	5%
Onisimus	Ve.	86%	3%	5%	3%	4%
Mesidotea	Unoiled	-	81%	83%	50%	71%
Mesidotea	N.W.	54%	39%	26%	21%	29%